UNIVERSITE MONTPELLIER 2- Habilitation à Diriger les Recherches

Protection intégrée des cultures cotonnières et horticoles en Afrique Sub-saharienne : De l'agrochimie à l'écologie chimique.

Thibaud MARTIN

CIRAD PERSYST, UPR HORTSYS,

Icipe, African Insect Science for Food and Health, Nairobi, Kenya

Le 17 septembre 2013

Composition du jury :

Pr Anne Marie Cortesero, Université de Rennes
Dr Luc Belzunces, INRA
Dr Didier Fontenille, IRD
Dr Samuel Nibouche, Cirad
Dr Mylène Weill, Université de Montpellier

A Armelle, Hugo, Hadrien et Alice

Et à tous ceux avec qui j'ai eu plaisir à travailler

Table des matières

1 Curriculum vitae

Etat civil
Français, né le 10/10/1964 à Rouen, Marié, 3 enfants

Adresse professionnelle
Icipe - African Insect Science for Food and Health - PO Box 30772-00100 Nairobi, Kenya Tel: +254 208 632 256 Mob: +254 708 725 683 ; Adresse électronique : thibaud.martin@cirad.fr & tmartin@icipe.org

Profession
Chercheur au CIRAD, Département PERSYST Performances des systèmes de production et de transformation tropicaux, UPR103 Hortsys 'Fonctionnement agroécologique et performances des systèmes de culture horticoles'

Expertise

Disciplines : Entomologie, Chimie, Agronomie ; Sciences de l'environnement

Filières : Horticole et Coton

Thématiques : IPM, Lutte physique ; Ecotoxicologie ; Ecosystèmes cultivés tropicaux ; Ecologie chimique

Activités : Recherche, Développement et Formation

Autres expertises

Innovation dans les systèmes de production ; Risques environnementaux, évaluations environnementales, impact des systèmes de production sur l'environnement

Expertise pays

Afrique Centrale, Afrique de l'Ouest, Afrique de l'Est et en particulier Tchad, Cameroun, Bénin, Burkina Faso, Côte d'Ivoire et Kenya

Principales qualifications

Gestion intégrée des populations d'arthropodes dans les systèmes de culture cotonniers et horticoles ; Lutte chimique et physique ; Caractérisation des mécanismes de résistance des insectes aux insecticides ; Lutte contre les insectes vecteurs de maladies humaines ; Ecotoxicologie ; Ecologie chimique

Cursus universitaire

2003 Doctorat de Chimie Université de Toulouse

1988 DEA Agrochimie Université de Toulouse

1987 Maîtrise Chimie Université de Toulouse

1986 Licence Chimie Université de Toulouse

1985 DEUG Biologie Université de Toulouse

Distinction et société savante

Prix de la recherche de Côte d'Ivoire, en collaboration avec Dr Ochou pour notre travail intitulé : Gestion de la Résistance de *Helicoverpa armigera* (Hübner) aux insecticides en culture cotonnière de Côte d'Ivoire. Décerné le 14/09/2005 par le Ministère de la Recherche Scientifique de Côte d'Ivoire

Membre de l'African Association of Insect Scientist (AAIS)

Expérience Professionnelle

08/2012 : Cirad Hortsys, Visiting scientist à Icipe - African Insect Science for Food and Health, Plant Health Dpt, Nairobi, Kenya

Coordination des projets BioActiveNet, BioNetAgro et NatProtect
Encadrement de 3 doctorants inscrits à l'ED Sibaghe en 2012

09/2009 - 07/2012 : Cirad PERSYST UPR Hortsys, Montpellier, France

PI projet BioActiveNet financé par A to Z Int. Mills Ltd (2011-2013) France, Kenya
PI projet NatProtect financé par la Fondation Mutavie (2011-2014) France, Kenya
Co-PI projet BioNetAgro financé par Hort CRSP USAID (2010-2013) USA et Cirad, France, Kenya et Bénin
Recherche sur les méthodes de lutte physique pour la protection des cultures horticoles ; Etude du comportement des ravageurs à l'égard de filets anti-insectes traités ou non avec des biocides ; Etude des interactions insectes / santé / agriculture dans les systèmes horticoles du Bénin et du Kenya

08/2004 - 08/2009 : IRD-UR016 Vector Control, Cotonou, Bénin

Co-PI projet Corus 6015 Impact des traitements agricoles sur les vecteurs du paludisme dans les zones cotonnières d'Afrique de l'Ouest ; Etude de l'impact des traitements agricoles sur la sélection des populations de vecteurs du paludisme résistants aux insecticides donc à l'interface santé/agriculture. Développement de nouveaux partenariats, montage de projets de recherche, enseignement au Master International d'Entomologie Médicale et Vétérinaire et participation aux projets de recherche.

10/2002 - 07/2004 : CIRAD-UMR CBGP - Montpellier France

Etude des mécanismes de résistance des insectes aux insecticides ; Soutenance de thèse en 2003

09/1996 - 09/2002 : CIRAD CNRA, Programme coton, Bouaké, Côte d'Ivoire

Chargé de la gestion des arthropodes dans les agro-systèmes à base coton de la Côte d'Ivoire ; Thèse sur l'étude de la résistance de *Helicoverpa armigera* aux insecticides. Elaboration de programmes de protection pour gérer ce phénomène ; Montage et animation scientifique du projet régional PR-PRAO

11/1994 - 08/1996 : CIRAD-CA - Montpellier France

Responsable du laboratoire d'entomophages et d'entomopathogènes du programme coton

07/1989 - 10/1994 : CIRAD-CA - Bebedjia Tchad

Chef de la station de recherche du CIRAD-CA de Bebedjia, Gestion et animation de la recherche (1994)

Gestion intégrée des populations d'arthropodes nuisibles et utiles dans les agro-systèmes à base coton du Tchad ; Recherche-développement en milieu contrôlé et milieu réel.

2 Descriptif de carrière

Recruté au Cirad en CDI en décembre 1989 après 2 stages de 6 mois, ma carrière d'entomologiste agricole s'est déroulée en quatre étapes : 1) 5 ans ingénieur de recherche au Tchad sur la station de recherche coton de Bébédjia (Cirad-IRCT) puis 1 an chef de station; 2) 6 ans ingénieur de recherche en Côte d'Ivoire au programme coton du Centre National de Recherche Agronomique de Bouaké où j'ai réalisé ma thèse de doctorat ; 3) 5 ans chercheur au Bénin en poste d'accueil à l'IRD-UR 16, affecté au Centre de Recherche Entomologique de Cotonou ; 4) 4 ans chercheur de l'UPR Hortsys au Cirad à Montpellier ; 5) Visiting scientist à l'Icipe Plant Health Department à Nairobi au Kenya depuis aout 2012. Ces missions à l'étranger ont été entrecoupées de 2 séjours de 2 ans à Montpellier qui m'ont permis de valoriser mon travail de recherche, acquérir et compléter mes connaissances en biochimie, en biologie moléculaire et ces deux dernières années en écologie chimique et à monter de nouveaux projets.

J'ai débuté ma carrière au Tchad (1989-1994) affecté à la station de recherche du Cirad-IRCT à Bebedjia dans un contexte d'insécurité permanente (coup d'état en 1990) mais sous la direction d'Alain Renou, ingénieur agronome, qui m'a transmis sa passion de l'agriculture et de l'Afrique. Ma thématique de recherche portait sur la protection intégrée de la culture du coton et du niébé.

Ingénieur chimiste de formation j'ai acquis sur le terrain des compétences générales en agronomie et en entomologie[1]. J'ai mené des recherches en laboratoire et en station de recherche sur l'efficacité biologique des outils de lutte, les risques d'acquisition de résistance[2,3,4], les stratégies de gestion des ravageurs[5,6,7] et leurs impacts sur les insectes utiles[8]. En collaboration avec mes collègues du Cirad et de la recherche Tchadienne nous avons élaboré pour les petits producteurs des outils d'aide à la décision et des stratégies de protection adaptées à leur technicité pour réduire l'utilisation des pesticides[9] (produits biologiques, seuils de tolérance, résistance variétale) et prévenir les risques de sélectionner des populations résistantes (stratégies d'alternance et de mosaïque). Les résultats de ces travaux ont été diffusés à travers des publications, des communications dans les conférences internationales sur le coton et lors des réunions annuelles du réseau des entomologistes du Cirad et des Centres Nationaux de Recherche d'Afrique de l'Ouest et du Centre. J'ai été nommé chef de la station de recherche la dernière année de mon séjour au Tchad. J'en ai assuré la gestion comptable et financière, la

[1] Martin T. 1996. *Ouvrage*. Déprédateurs du cotonnier en Afrique tropicale et dans le reste du monde N°10, 44 p..
[2] Martin T. & Renou A. 1990. *Revue Scientifique du Tchad*, 1 (*2*) : 95-100
[3] Martin T. & Jacquemard P. 1991, *Coton et Fibres Tropicales*, 46 (*1*) : 5-13
[4] Martin T. Et al. 1992. *Coton et Fibres Tropicales.*, 48 (*4*) : 283-294
[5] Gopaye et al. 1992 *Revue Scientifique du Tchad*, 2 (*1-2*) : 83-92.
[6] Renou et al. 1993 *Coton et Fibres Tropicales.*, 48 (*2*) : 121-137.
[7] Martin et al. 1996 *Sahel IPM* (*8*) : 14-17.
[8] Sigrist et al. 1994. Réunion phytosanitaire de coordination cultures annuelles Afrique centrale, 1994-01-26/1994-01-29, Maroua, Cameroun.
[9] LeDiambo. et al. 1992 *Revue Scientifique du Tchad*, 2 (*1-2*) : 163-168.

gestion des ressources humaines (9 chercheurs et une centaine d'employés permanents et saisonniers), l'animation scientifique et la coordination des projets.

En Côte d'Ivoire (1996-2002) j'ai évolué du statut d'ingénieur de recherche à celui de chercheur dans un contexte budgétaire mal défini pour des raisons externes au Cirad lié à une affectation au Centre National de Recherche Agronomique (CNRA) en pleine restructuration et avec les contraintes d'une insécurité permanente en fin de séjour (6 coups d'états et un rapatriement).

Grâce aux contacts pris lors de mon séjour au Tchad mais aussi sur place grâce à mes compétences relationnelles, j'ai obtenu des financements de partenaires privés (Bayer, Arysta, Syngenta, CIDT) pour réhabiliter et équiper le laboratoire d'entomologie dont j'étais responsable. Ma thématique de recherche portait sur la résistance aux insecticides de la noctuelle du cotonnier *Helicoverpa armigera*, l'identification des mécanismes impliqués et l'élaboration d'une nouvelle stratégie de protection de la culture cotonnière pour gérer ce phénomène à l'échelle régionale. J'ai caractérisé la résistance aux insecticides[10,11], identifié le mécanisme[12,13] et évalué plusieurs stratégies pour contrer cette résistance[14]. En parallèle, nous avons évalué différentes stratégies de protection intégrée avec les agriculteurs[15]. J'ai noué des collaborations durables avec l'équipe de l'IRD-UR016 basée à l'Institut Pierre Richet et l'Université de Toulouse où j'ai fait mes études de cinétique enzymatique. Ce travail a été synthétisé dans une thèse de doctorat en chimie soutenue en 2003 à l'Université de Toulouse sous la direction du Pr Didier Fournier[16]. Ce travail, fruit d'une étroite collaboration avec le Dr Ochou, responsable de l'équipe coton du CNRA, a été récompensé par le Prix de la Recherche 2003 de Côte d'Ivoire qui nous a été décerné en 2004 à Abidjan par le Ministre de la Recherche et de l'Enseignement Supérieur de Côte d'Ivoire. Au cours de cette période j'ai aussi initié en 1998 puis animé jusqu'en 2002 le Projet Régional PR-PRAO basé sur un réseau d'entomologistes de 7 pays d'Afrique de l'Ouest et du Cirad qui a permis l'application dés 1998 d'une stratégie de protection du cotonnier à l'échelle régionale pour gérer la résistance de *H. armigera* aux pyréthrinoïdes. Cette stratégie basée sur la suppression des pyréthrinoïdes jusqu'au 15 aout, l'alternance des familles chimiques et une stratégie de traitement « en fenêtre » est toujours appliquée avec succès en 2010 par plus d'un million de petits planteurs et illustre l'intérêt d'un tel réseau Recherche/Développement pour les filières agricoles africaines[17,18].

Rapatrié de Côte d'Ivoire en septembre 2002, j'ai été détaché dans l'équipe Cirad de l'UMR CBGP « Centre de Biologie et de Gestion des Population » à Montpellier. J'ai participé aux études en cours dans le laboratoire de JM Vassal en particulier la recherche de mutations du gène *kdr* pouvant expliquer la résistance de *H. armigera* aux pyréthrinoïdes. J'ai aussi réalisé un contrat de recherche pour la société Makteshim-Agan sur la résistance aux insecticides des Meligethes du Colza. J'y ai acquis de nouvelles compétences en biologie moléculaire.

[10] Martin et al. 2000 *Pest Management Science* 56, 549-554.
[11] Martin et al. 2002a. *Resistance Pest Management Newsletter*, 12, 16-19.
[12] Martin et al 2002b. *Pesticide biochemistry and physiology*, 74, 17-26.
[13] Martin et al. 2003b. *Insect biochemistry and molecular biology*, 33, 883-887.
[14] Martin et al. 2003a. *Journal of economic entomology*, 96, 468-474.
[15] Ochou & Martin. 2002c. *Resistance Pest Management Newsletter*, 12, 10-16.
[16] Martin 2003 Thèse de doctorat de Chimie, Université de Toulouse III, 81 pp.
[17] Martin et al. 2005. *Agriculture, Ecosystems and Environment*, 107, 409–411
[18] Youm et al. 2005. Book edited by Hari C. Sharma. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, 470pp.

A la demande de l'IRD-UR016 «Caractérisation et contrôle des populations de vecteurs», j'ai élaboré un projet de recherche «Interactions Santé/Agriculture». J'ai ensuite été recruté par l'IRD sur un poste d'accueil et été affecté en août 2004 dans l'équipe de l'UR016 au Centre de Recherche Entomologique de Cotonou au Bénin (2004-2009). Ma problématique de recherche portait sur l'impact des traitements agricoles sur la sélection des populations de vecteurs du paludisme résistants aux insecticides. J'ai construit des collaborations avec des centres de recherche agronomiques (INRAB, IITA), de santé publique (CREC, ISBA), des Universités (Abomey Calavi, Lomé, Ouagadougou, Toulouse, Montpellier, Lyon) et une ONG (APRETECTRA). Pour répondre aux problèmes d'utilisation abusive des pesticides soulevés par la caractérisation des pratiques phytosanitaires des petits maraîchers[19], mes recherches ont abouti à la mise au point de techniques innovantes de protection des cultures maraîchères permettant de réduire significativement l'utilisation d'insecticide[20,21] ainsi que d'un indicateur biologique pour évaluer le risque de présence de résidus d'insecticide[22]. J'ai également poursuivi des études sur la résistance aux insecticides des ravageurs *H. armigera*[23], d'*Aphis gossypii*[24] et des vecteurs de maladie *Aedes aegypti* et *Anopheles gambiae*[25]. J'ai acquis de nouvelles compétences en entomologie médicale en collaborant aux activités de l'UR016 portant sur l'évaluation des moyens de lutte contre les moustiques[26,27], les expertises pour l'OMS, l'encadrement d'étudiants et l'enseignement universitaire (Master MIE). Pour financer mes activités de recherche j'ai monté et coordonné un projet national (WB Development Market Place 2005) et régional comme le projet CORUS 6015 qui a permis de financer les recherches de 3 doctorants et d'un post doctorant sur des sujets portant sur la résistance aux insecticides des populations de *Bemisia tabaci*[28,29] et d'*An. gambiae*[30,31,32] dans les zones cotonnières et horticoles du Bénin, du Togo et du Burkina Faso. Ces études ont montré la résistance de *B. tabaci* aux pyréthrinoïdes, OPs et néonicotinoïdes d'une part et mis en évidence de nouveaux mécanismes de résistance aux pyréthrinoïdes et aux OPs dans les populations anopheliennes en particulier dans les zones où les traitements phytosanitaires sont les plus fréquents[33]. Ces travaux ont également montré la présence d'un biotype invasif de *B. tabaci* (Q1) d'origine méditerranéenne dans les populations collectés sur coton et vivant en sympatrie avec le biotype local (ASL) au Burkina Faso et dans le nord du Togo et du Bénin[34,35].

Suite aux résultats encourageants en terme de réduction de traitement insecticide avec les filets anti-insectes et les filets acaricides pour protéger les cultures maraîchères au Bénin, j'ai intégré l'UR Hortsys du Cirad en avril 2009 pour développer ces techniques et les adapter aux cultures

[19] Ahouangninou et al. 2011. *Cahiers de l'Agriculture,* 20, 216-22
[20] Martin et al. 2006. *Journal of Economic Entomology*, 99, 450-454.
[21] Licciardi et al. 2008. *International Journal of Tropical Insect Science*, 27, 152-158.
[22] Martin et al. 2007. *Tropical Medicine and International Health*, 12, 2, 1-6.
[23] Achaleke et al. 2009. *Pest Management Science* 65, 1147-1154.
[24] Carletto et al. 2009. *Pest Management Science* 66, 301-307.
[25] Corbel V. et al. 2007. *Acta Tropica*, 101, 207-216.
[26] Hougard, J. M. et al. 2007. *Journal of Medical Entomology*, 44, 651-655.
[27] Pennetier C. et al. 2010. *Parasites & Vectors*. N° 1086899143405058
[28] Houdete et al. 2010. *Pesticide Biochemistry and Physiology, 98, 115-120*.
[29] Houndete et al. 2010. *Pest Management Science* 66: 1181–1185
[30] Djegbe et al. 2011. *Malaria Journal, 10, 261*.
[31] Namountougou et al. 2012. *PlosOne*, 7,11.
[32] Namountougou et al. 2012. *PlosOne*, 7,11.
[33] Yadouleton et al. 2011. *Parasites & Vectors* 4:60
[34] Gnankine et al. 2012. *Insect Conservation and Diversity*
[35] Gnankine et al. 2013. *Insect Journal of Tropical Insect Science*

et aux agrosystèmes des zones urbaines et péri-urbaines d'Afrique subsaharienne. En octobre 2010 le projet de recherche BioNetAgro (2010-2013) basé sur l'utilisation de filets anti-insectes pour la protection des cultures maraîchère, élaboré avec le Pr Mathieu Ngouadjio de l'Université de l'Etat du Michigan (MSU), a été financé par le département Horticulture de l'USAID (Hort CRSP) pour un montant de 500 KUS$ complété de 210 K€ par le CIRAD. En 2011 et 2012 j'ai obtenu un financement (2 x 50 K€) de la société tanzanienne A to Z Textile Mills Ltd dans le cadre d'un MOU signé avec le Cirad pour étudier l'effet des filets répulsifs sur le comportement de 4 ravageurs majeurs : *Plutella xylostella*, *B. tabaci*, *Myzus persicae* et le thrips *Frankliniella occidentalis*. J'ai obtenu aussi 196 K€ de la fondation Mutavie pour financer le projet NatProtect intégrant une bourse de thèse pour rechercher et étudier les effets de produits naturels permettant de renforcer/compléter l'efficacité des filets anti-insectes. En 2012 j'ai obtenu le financement de 2 autres bourses de thèse : l'une par le Cirad (bourse thèse du Sud) sur l'étude du comportement de l'acarien invasif *Tetranychus evansi* sur tomate et son contrôle sous abris, et une autre financée par le programme AgTrain de l'UE sur l'élaboration d'une stratégie IPM contre le thrips du niébé *Megalurothrips sjostedti* au Kenya. Enfin j'ai intégré en aout 2012 le département Santé des Plantes de l'Icipe à Nairobi au Kenya en tant que visiting scientist afin de pouvoir utiliser leur plateforme d'écologie chimique pour étudier les interactions insectes/ravageurs/plantes, suivre au plus près les expérimentations au champs conduites en station de recherche avec le KARI et l'Université d'Egerton, et discuter avec les agriculteurs des avantages et inconvénients de la lutte physique.

3 Liste des publications

3.1 Thèse

Thibaud Martin 2003. La résistance de *Helicoverpa armigera* Hubner aux insecticides en Afrique de l'Ouest : Du mécanisme à la gestion des populations. Thèse de doctorat en chimie soutenue en 2013 à l'Université de Toulouse sous la direction du Pr Didier Fournier

3.2 Publications scientifiques dans des revues à facteur d'impact

33- Martin T, R Palix, A Kamal, E Delétré, R Bonafos, S Simon, M Ngouajio 2013. A repellent treated netting as a new technology for protecting vegetable crops. *Journal of Economic Entomology* (accepté)

32- Tia EV, AA Adima, AJ Gnago, SL Niamké, T Martin, C Menut, P Lozano, Y Lozano 2013. Fumigant toxicity of *Melaleuca leucadendron* L. and *Hyptis suaveolens* L. essential oils against *Plutella xylostella* L. (Lepidoptera: Yponometidae). *Journal of Environmental Protection* (accepté)

31- Gnankiné O, G Ketoh, T Martin 2013. Dynamics of Mediterranean species (MED) of the *Bemisia tabaci* species complex in two wester African countries. *International Journal of Tropical Insect Science* (accepté)

30- Gnankiné O, L Mouton, A Savadogo, T Martin, A Sanon, RK Dabire, F Vavre, F Fleury 2013. Biotype status and resistance to neonicotinoids and carbosulfan in *Bemisia tabaci*

(Hemiptera: Aleyrodidae) in Burkina Faso, West Africa. *International Journal of Pest Management* DOI:10.1080/09670874.2013.771806.

29- Ahouangninou C, T Martin, F Assogba-Komlan, S Simon, L Djogbénou, I Siddick, C Pennetier, V Corbel, B Fayomi 2013. Using *Aedes aegypti* larvae to assess pesticide contamination of soil, groundwater and vegetables. *British Biotechnology Journal*, 3,2: 143-157.

28- Muleke EM, M Saidi, FM Itulya, T Martin, M Ngouajio 2013. Enhancing Cabbage Seed Germination and Seedling Quality Using Eco-Friendly Nets. *Agronomy* 3, 2. (www.mdpi.com/journal/agronomy)

27- Namountougou M, Diabaté A, Etang J, Toé KH, Sawadogo S, Baldet T, Martin T, Chandre F, Simard F, Dabiré KR 2012. First report of L1014S *kdr* mutation in wild populations of *Anopheles gambiae* M and S molecular forms in Burkina Faso (West Africa). *Acta Tropica* 125, 123–127. http://dx.doi.org/10.1016/j.actatropica.2012.10.012

26- Tia E. V., P. Lozano, C. Menut, Y.F. Lozano, T. Martin, S. Niamké, A. A. Adima, Potentialité des huiles essentielles dans la lutte biologique contre la mouche blanche *Bemisia tabaci* Gen. *Phytothérapie*. http://dx.doi:10.1007/s10298-012-0736-8

25- Namountougou M, F Simard, T Baldet, A Diabaté, O Gnankiné, JB Ouédraogo, T Martin, RK Dabiré 2012. Multiple Insecticide Resistance in *Anopheles gambiae* s.l. populations from Burkina Faso, West Africa. *PLoS ONE* 7(11): e48412. http://dx.doi:10.1371/journal.pone.0048412

24- Gogo EO, M Saidi, FM Itulya, T Martin, M Ngouajio 2012. Microclimate Modification Using Eco-Friendly Nets for High Quality Tomato Transplant Production by Small-Scale Farmers in East Africa. *Hort Technology*, 22, 292-298.

23- Gnankiné O, L Mouton , H Henri, T Houndeté , T Martin, F Vavre, F Fleury 2012. Distribution of the biotypes of Bemisia tabaci (Homoptera: Aleyrodidae) and their associated symbiotic bacteria on host plants in western Africa. *Insect Conservation and Diversity*. http://dx.doi: 10.1111/j.1752-4598.2012.00206.x

22- Ahouangninou CCA, T Martin, P Edorh, IA Siddick, S Bio-Bangana, S Dion, O Samuel, L St-Laurent, M Boko, S Simon, BE Fayomi 2012. Characterization of health and environmental risks of pesticide use in market-gardening in the rural city of Tori-Bossito in Benin, West Africa. *Journal of Environmental Protection*, 3, 241-248. http://dx.doi.org/10.4236/jep.2012.33030

21- Djègbè I, O Boussari, A Sidick,T Martin, H Ranson, F Chandre, M Akogbéto, V Corbel 2011. Dynamic of insecticide resistance in malaria vectors in Benin: First evidence for the presence of *L1014S* kdr mutation in *An. gambiae* from West Africa. *Malaria Journal*, 10:261. http://dx.doi:10.1186/1475-2875-10-261

20- Tia EV, AA Adima, SL Niamké, AJ Gnago, T Martin, P Lozano, C Menut 2011. Chemical Composition and Insecticidal Activity of Essential oils of two Aromatic plants from Ivory Coast against *Bemisia tabaci* G. (Hemiptera: Aleyrodidae). *Natural Product Communication* 6(8):1183-8.

19- Ahouangninou C, B Fayomi, T. Martin 2011. Évaluation des risques sanitaires et environnementaux des pratiques phytosanitaires des producteurs maraîchers dans la commune rurale de Tori-Bossito (Sud-Bénin). *Cahiers de l'Agriculture* 20 : 216-22. http://dx.doi: 10.1684/agr.2011.0485

18- Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbenou L, Dabiré R, Aïkpon R, Boko M, Glitho I, Akogbeto M 2011. Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin. *Parasites & Vectors,* **4,** 60. http://dx.doi:10.1186/1756-3305-4-60

17- Pennetier C, J Chabi, T Martin, F Chandre, C Rogier, JM Hougard, F Pages 2010. New protective battle-dress impregnated against mosquito vector bites. *Parasites & Vectors*, 3, 81, 7 p. http://dx.doi.org/10.1186/1756-3305-3-81

16- Houndété TA, GK Kétoh, OSA Hema, T Brévault, IA Glitho, T Martin 2010. Insecticide resistance in field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in West Africa. *Pest Management Science* 66: 1181–1185. http://dx.doi:10.1002/ps.2008

15- Houndété TA, D Fournier, GK Ketoh, IA Glitho, R Nauen, T Martin 2010. Biochemical determination of acetylcholinesterase genotypes conferring resistance to the organophosphate insecticide chlorpyriphos in field populations of *Bemisia tabaci* from Benin, West Africa. *Pesticide Biochemistry and Physiology*, 98, 115-120. http://dx.doi:10.1016/j.pestbp.2010.05.009

14- Brun-Barale A, O Héma, T Martin, S Suraporn, P Audant, H Sezutsu, R Feyereisen 2010. Multiple P450 gene overexpressed in deltamethrin-resistant strains of *Helicoverpa armigera*. *Pest Management Science* 66, 900-909. http://dx.doi.org/10.1002/ps.1960

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1997- Martin T., Ochou G.O., Hala N'Klo F., Vassal J.M., Vaissayre M.. Perte de sensibilité aux pyréthrinoïdes chez *Helicoverpa armigera* (Huebner) en Côte d'Ivoire. In *Réunion phytosanitaire de l'Afrique de l'Ouest et du Centre/CORAF; CIRAD-CA. - Abidjan : CORAF, 1997*, p.253-261. Réunion phytosanitaire de l'Afrique de l'ouest et du centre, 1997-01-27/1997-01-31, Cotonou, Bénin

1997- Vassal J.M., Martin T., Hala N'Klo F., Ochou G.O., Vaissayre M.. Decrease in the susceptibility of *Helicoverpa armigera* (Huebner) to Pyrethroid insecticides in Côte d'Ivoire. In *Resistance'97/IDESSA; CIRAD. - Montpellier : CIRAD, 1997*, .[3 p.)] p. Resistance Conference of Rothamsted, 1997-04-14/1997-04-16, Harpenden, Royaume-Uni

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1994- Bedingam Le Diambo M., Martin T., Renou A.. Approche régionale des problèmes phytosanitaires de la culture du niébé en zone soudanienne au Tchad. In *Réunion phytosanitaire de coordination cultures annuelles. Afrique centrale = [Coordination plant protection meeting annual crops. Central Africa]/CIRAD-CA; IRA. - Montpellier : CIRAD-CA, 1994*, p.190-193. Réunion phytosanitaire de coordination cultures annuelles Afrique centrale, 1994-01-26/1994-01-29, Maroua, Cameroun

1994- Sigrist J.C., Martin T., Renou A. Effets non intentionnels des pesticides sur l'entomofaune utile des cotonniers. In *Réunion phytosanitaire de coordination cultures annuelles. Afrique centrale = [Coordination plant protection meeting annual crops. Central Africa]/CIRAD-CA; IRA. - Montpellier : CIRAD-CA, 1994*, p.154-175. Réunion phytosanitaire de coordination cultures annuelles Afrique centrale, 1994-01-26/1994-01-29, Maroua, Cameroun

1991- Martin T. Synthèse des résultats phytosanitaires des Etats d'Afrique Centrale. In *Réunion de coordination de recherche phytosanitaire cotonnière/Toe A. (ed.), Nibouche S. (ed.), Pinchard V. (ed.). - Paris : CIRAD-IRCT, 1991*, p.24-35. Commission régionale défense des cultures Afrique de l'ouest CIRAD-IRCT, réseau coton coraf, INERA. 7, 1991-01-26/1991-01-31, Ouagadougou, Burkina

4 Liste des thèses et stages encadrés et formations dispensées

4.1 Encadrement de thèses

2012-2015 : Andnet Bayleyegn Abtew (Ethiopien) A study of the behavior, ecology and control of the legume flower thrips, *Megalurothrips sjostedti* (Trybom) in beans towards the development of an integrated pest management (IPM) program in Kenya. Thèse de doctorat financée par le programme AgTrain de l'Union européenne démarrée en octobre 2012 au Cirad, Montpellier et poursuivie à Icipe depuis novembre 2012- Thèse en Biologie Animale, ED Sibaghe, Université de Montpellier, Directeur Pr Serge Kreiter, Directeur Dr Giovanna Tropea Garzia Catania University, co-directeur et encadrant Dr Thibaud Martin

2012-2015 : Ginette Azandeme (Béninoise) Development of an original technique to fight against phytophagous mites. Environmental impact and complementarity with other methods in protecting horticultural crops. Thèse de doctorat financée par CIRAD démarrée en février 2012 au Cirad, Montpellier et poursuivie à Icipe depuis from juin 2012. Thèse en Biologie Animale, ED Sibaghe, Université de Montpellier, Directeur Pr Serge Kreiter, co-directeur et encadrant Dr Thibaud Martin

2011-2014 : Emilie Delétré (Française) Etude du comportement de deux insectes vecteurs de maladie à l'égard de filets imprégnés de produits naturels, pour un usage en santé humaine et en agriculture. Thèse de doctorat financée par la foundation Mutavie, démarrée en décembre 2011 au Cirad, Montpellier et poursuivie à Icipe depuis from juin 2012. Thèse en Biologie Animale, ED Sibaghe, Université de Montpellier, Directeur Dr Denis Bourguet, co-directeur et encadrant Dr Thibaud Martin (2 articles en préparation, 1 soumis)

2009-2013 : Claude Ahouangninou (Béninois) Analyse de la dynamique de l'impact environnemental et sanitaire de la production maraîchère urbaine au Bénin. Thèse de doctorat démarrée en janvier 2009 à l'Institut des Sciences Biomédicales Appliquées, Cotonou. Soutenance prevue en mars 2013. Thèse en Environnement, Université d'Abomey Calavi Bénin, Directeur Pr Benjamin Fayomi, co-directeur et encadrant Dr Thibaud Martin (3 articles publiés)

2007-2010 : Thomas Houndete (Béninois) Résistance de *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) aux insecticides en Afrique de l'Ouest. Thèse de doctorat financée par SCAC Cotonou et projet Corus, démarrée en septembe 2007 à INRAB, Abomey Calavi et poursuivie à IRD-CREC, soutenue en septembre 2010. Thèse en Entomologie soutenue le 30/09/2010 à l'Université de Lomé, Togo, Directeur Pr Isabelle A. Glitho, co-directeur et encadrant Dr Thibaud Martin (2 articles publiés)

4.2 Encadrement de stage

2013 Claire Dumenil (Française) Etude du comportement du moustique *Anopheles gambiae* à l'égard de moustiquaires imprégnées des composés majoritaires de 5 huiles essentielles répulsives. Co-encadrement avec Fabrice Chandre et Emilie Delétré.

M2 : Amsterdam University

2012 Barbara Barkman (Neerlandaise) Evaluation biologique de différents filets imprégnés de produit naturels contre la mouche blanche *Bemisia tabaci*, ravageur majeur des cultures horticoles. Co-encadrement avec Emilie Delétré.

M2 : Amsterdam University

2012 Aurélien Puiseux (Français) Evaluation biologique de différents filets imprégnés de produit répulsifs contre les Thrips *Frankliniella occidentalis*, ravageur majeur des cultures horticoles

M1 : Ecole Normale Supérieure

2012 Miriam Kungu (Kenyane) Influence of circadian migration behaviour of *Tetranychus evansi* and its predator, *Phytoseiulus longipes* on the efficacy of acaricide treated net in the management of the red spider mite on African black nightshade. Co-encadrement avec Sevgan Subremanian et Komi Fiaboe.

Msc : Jomo Kenyatta University and Icipe

2012 Maelle Mallent (Française) Evaluation biologique de filets imprégnés de différents produits répulsifs naturels contre un ravageur majeur des cultures horticoles: la mouche blanche *Bemisia tabaci*.

L3 : Université de Montpellier

2012 Andy Cadin (Français) Evaluation biologique de 20 huiles essentielles sur *Anopheles gambiae* vecteur du paludisme.

L3 : Université de Montpellier

2012 Gaelle Le Dilosquer (Française) Evaluation biologique d'une nouvelle méthode de protection des cultures horticoles contre les acariens phytophages, basée sur l'utilisation de matériaux imprégnés d'acaricide.

Ingénieur Agronome : Agrocampus Ouest

2011 Emilie Delétré (Française) Evaluation biologique d'une nouvelle méthode de protection des cultures horticoles contre les acariens phytophages basée sur l'utilisation de matériaux imprégnés d'acaricide.

Ingénieur Agronome : Agrocampus Ouest (1 article soumis)

2011 Romain Palix (Français) Evaluation biologique de différents filets de protection des cultures contre les pucerons sur chou et leurs parasites.

M2 : Université de Tour (1 article soumis)

2011 Aldi Kamal (Indonesien) Evaluation biologique de différents filets de protection des cultures contre Bemisia tabaci et ses parasites.

M2 : Université de Montpellier (1 article soumis)

2010 Hubert Kacou Koffi (Ivoirien) Evaluation biologique et agronomique de différents filets anti-insectes contre la teigne du chou *Plutella xylostella*

M2: SupAgro France (1 article en préparation)

2009 Adama Zida (Burkinabé) Etude de la résistance au carbosulfan (carbamate) d'Anopheles gambiae au Bénin

M2 MIE : UM2 & Université d'Abomey Calavi Bénin

2009 Elinam Ami Dadzie (Togolaise) Impact des fertilisants sur l'oviposition et le développement larvaire chez Anopheles gambiae en milieu urbain ouest-africain.

M2 MIE: UM2 & Université d'Abomey Calavi Bénin

2008 Claude Ahouangninou (Béninois) Etude des pratiques phytosanitaires maraîchères et évaluation de leur risque sur la santé et l'environnement dans la commune de Tori-bossito au sud Bénin

M2 Environnement: Université d'Abomey Calavi Bénin (1 article publié)

2008 Moussa Cisse (Malien) Caractérisation de la résistance aux insecticides des populations Anophéliennes de Cotonou au Bénin

M2 MIE: UM2 & Université d'Abomey Calavi Bénin (1 article en préparation)

2007 Séverine Licciardi (Française) Etude du comportement d'*Anopheles gambiae* en présence d'un dormeur sous moustiquaire.

M2 MIE: UM2 & Université d'Abomey Calavi Bénin

2006 Séverine Licciardi (Française) Etude de l'efficacité des filets anti-insectes pour protéger les cultures maraîchères au Bénin.

Volontaire Internationale (1 an) (1 article publié)

2004 Nadia Lubin (Française) Étude des mécanismes de résistance aux insecticides des Méligethes du colza et efficacité des mélanges insecticides pour contrer cette résistance

Brevet de Technicien Supérieur Agricole Option Technologies végétales, Fort de France, France

2003 Julien Pompon (Français) Recherche des mutations sur le gène *kdr* chez des populations africaines de *Helicoverpa armigera*.

DESS : Université Montpellier 2, France

2003 Omer Hema (Burkinabé) caractérisation des modifications enzymatiques liées aux mécanismes de résistances aux insecticides chez *Helicoverpa armigera*

DEA : Université de Ouagadougou, Burkina Faso

2000 Patrick A. Yobo-Titiahy (Ivoirien) Evolution de la résistance de *Helicoverpa armigera* collectée dans différents systèmes de cultures en Côte d'Ivoire

Ingénieur agronome : Institut National Polytechnique, Yamoussoukro, Côte d'Ivoire

2000 Denise Youbou (Ivoirienne) Efficacité comparée des nouvelles familles chimiques sur *Helicoverpa armigera* résistant aux pyréthrinoïdes et leur action conjointe avec un organophosphoré

Ingénieur agronome : Institut National Polytechnique, Yamoussoukro, Côte d'Ivoire

1999 Alain M.J. Ehouman (Ivoirien) Caractérisation de la résistance de Helicoverpa armigera (Hübner, 1808) aux insecticides sur le cotonnier en Côte d'Ivoire

Ingénieur agronome : Institut National Polytechnique, Yamoussoukro, Côte d'Ivoire

1998 Anouck Oechsner de Coninck (Française) Evaluation du devenir d'un insecticide, le Deltaphos, dans un agro-système cotonnier de Côte d'Ivoire

Ingénieur agronome : ENSAM, France

1998 G.F. Gnaore (Ivoirien) Sensibilité de *Helicoverpa armigera* (Hübner, 1808) aux insecticides chimiques en Côte d'Ivoire et étude d'un mécanisme de résistance

Ingénieur agronome : Institut National Polytechnique, Yamoussoukro, Côte d'Ivoire

1998 Thierry Tafforeau (Français) Etude d'un nouveau ravageur du cotonnier en Côte d'Ivoire *Scirtothrips dorsalis* Hood.

Ingénieur agronome : ENSAM, France

1994 Jean Charles Sigrist (Français) Effets non intentionnels des pesticides sur l'entomofaune utile des cotonniers

Ingénieur agronome : ISTOM, France

4.3 Formations dispensées

- Master International d'Entomologie Médicale et Vétérinaire des Universités de Montpellier 2 et d'Abomey Calavi (2005-2011) : Cours théorique (3h) sur l'impact des traitements agricoles sur la sélection des vecteurs du paludisme et TD (1h) sur les tests enzymatiques pour mesurer l'activité des estérases chez les insectes ;
- Master d'Entomologie Appliquée de l'Université d'Abomey Calavi (2006-2008) : Cours théorique (5h) sur la résistance des insectes aux insecticides ;
- Master Hortimet de l'Université de Montpellier 2 (2009-2011): cours théorique (3h) sur la protection physique des cultures maraîchères en zone tropicale ;

5 Bilan des activités de recherche

Mes activités de recherche se situent essentiellement dans le domaine de la protection des cultures cotonnières et horticoles en Afrique de l'Ouest. La lutte chimique y est de loin la plus pratiquée par les petits agriculteurs pour des raisons de coût, de disponibilité et de facilité d'utilisation. L'objectif principal de mes recherches a été de réduire l'utilisation de ces insecticides chimiques potentiellement nocifs pour la santé des populations et leur environnement. Pour atteindre cet objectif mes recherches ont porté sur l'identification des pratiques phytosanitaires des agriculteurs, la résistance aux insecticides des principaux ravageurs, l'impact de ces insecticides sur la santé des populations et leur environnement et enfin l'élaboration de stratégies alternatives, adaptées à la technicité des agriculteurs, permettant de garantir une production de qualité avec le plus faible impact environnemental possible.

En Afrique Subsaharienne la lutte chimique est principalement utilisée par les petits producteurs pour la protection des cultures cotonnières et maraîchères. Ces cultures sont complémentaires pour plusieurs raisons : 1) le coton est cultivé en saison des pluies et les cultures maraîchères toute l'année mais surtout en saison sèche; 2) le coton est cultivé sur de grandes superficies en zone soudano-sahelienne, les cultures maraichères sur de petites parcelles le long des fleuves Niger et Sénégal mais aussi et surtout dans les zones urbaines et périurbaines ; 3) la culture du coton est fortement structurée et encadrée par l'état, la pratique du maraîchage est informelle et la filière est très peu organisée ; 4) la fibre de coton est majoritairement exportée tandis que la production maraîchère est consommée localement. Cependant deux ravageurs parmi les plus dangereux passent d'un système de culture à l'autre : la noctuelle de la tomate, *Helicoverpa armigera* et l'aleurode *Bemisia tabaci.* Les chenilles d'*H. armigera* sont extrêmement voraces tandis que l'aleurode est un vecteur potentiel de phytovirus particulièrement dommageables pour les solanacées. Et, comme nous le verrons par la suite, les formulations insecticides utilisées passent aussi d'un système à l'autre.

5.1 Une stratégie de gestion de la résistance en culture cotonnière

En Afrique de l'Ouest, la culture cotonnière est pratiquée bon an mal an par environ deux millions d'agriculteurs, qui lui consacrent un hectare en moyenne (CSAO/OCDE, 2005). C'est la plus importante culture de rente de la région soudano-sahélienne car elle procure plus de 50% des ressources financières de la population rurale (en moyenne de l'ordre de 150 € par hectare et par an). Les exportations de l'Afrique de l'Ouest (3ième exportateur mondial) ont atteint 800.000 tonnes de fibre en 2006 pour une valeur de 890 millions $ US. Environ 16 millions de personnes sont impliquées dans la production, la transformation et la commercialisation. Elle contribue largement à la lutte contre la pauvreté par son effet d'entraînement sur le développement agricole en général (Ministère de la coopération, 1991).

Le cotonnier est attaqué par plus d'une dizaine de ravageurs, comprenant plusieurs espèces de lépidoptères, des insectes piqueurs-suceurs (pucerons, aleurodes, punaises) et des acariens. De nombreuses stratégies de protection ont été élaborées au cours des cinquante dernières années

pour lutter contre leurs infestations sur des bases biologiques, chimiques et mixtes[36,37]. Mais seules les moins coûteuses et les plus faciles à vulgariser ont été développées à grande échelle par les sociétés cotonnières Africaines étatiques ou privées. Cette stratégie de lutte intégrait des pratiques culturales appropriées et des variétés présentant certains traits de tolérance aux insectes, en complément desquelles quatre à six traitements insecticides étaient appliqués systématiquement à 14 jours d'intervalle depuis la floraison pour obtenir une production moyenne de plus d'une tonne de coton-graine/ha. Ceci dit les agriculteurs revendent ou détournent souvent sur d'autres cultures, une partie des insecticides obtenus à crédit (Fadoegnon *et al.* 2003). A juste titre d'ailleurs car, là où elle a été pratiquée, la formation des agriculteurs à la reconnaissance des ravageurs a permis de réduire en moyenne de 50% les doses d'insecticides appliquées dans le cadre d'une stratégie de « lutte étagée ciblée » où la décision de traitement est prise selon le niveau d'infestation des principaux ravageurs ou leurs dégâts apparents (Silvie *et al.* 2013). Au Mali, par exemple, la lutte étagée ciblée est diffusée depuis 1994 et elle représentait 29 % des surfaces cotonnières en 2008.

Au début des années 90, de fortes infestations de chenilles de l'espèce *Helicoverpa armigera* ont été observées dans tous les pays cotonniers d'Afrique de l'Ouest. Elles sont devenues d'année en année toujours plus précoces, plus fréquentes et toujours plus difficiles à contrôler, entraînant le mécontentement des producteurs se plaignant de l'inefficacité des insecticides malgré l'augmentation des doses et des fréquences de traitement. L'hypothèse d'une résistance aux insecticides était d'autant plus plausible que dans d'autres pays cotonniers des résistances aux pyréthrinoïdes avaient été observées chez cette Noctuelle. Dans des systèmes de culture comparables conduits par des petits producteurs et avec un cortège de ravageurs identique, la résistance des insectes aux insecticides (de *H. armigera* et d'autres ravageurs) ont conduit les petits planteurs de coton indiens à effectuer en moyenne 20 à 40 traitements à des doses 2 à 3 fois plus fortes qu'en Afrique de l'Ouest pour une production 2 fois plus faible d'environ 500 kg/ha de coton graine (Kranthi *et al.* 2002).

Face à ce danger, les pays cotonniers d'Afrique de l'Ouest ont conjugué leurs efforts et accepté des entomologistes coton du Cirad et des Centres Nationaux de Recherche le financement d'un réseau régional pour la Prévention et gestion de la Résistance aux insecticides en Afrique de l'Ouest (PRAO). Les études de terrain ont permis de localiser les populations résistantes présentes dans les deux bassins de production, Togo/Bénin et Burkina Faso/Côte d'Ivoire/Mali, mais à des niveaux variables[38]. Parallèlement dans le cadre de ma thèse mes travaux en laboratoire ont révélé des facteurs de résistance élevés suite à une pression de sélection à la deltaméthrine (100 fois par rapport à une souche sensible), expliquant l'inefficacité des traitements avec des pyréthrinoïdes[39]. Mes résultats ont aussi montré que les chenilles résistantes aux pyréthrinoïdes présentaient une activité enzymatique, en particulier des oxydases (ou cytochrome P450), plus importante que la souche sensible de référence[40]. Des études similaires effectuées par la suite sur des populations de *H. armigera* collectées au Cameroun ont aussi montré une résistance métabolique liée à une surproduction d'estérases[41]. Finalement, les

[36] Gopaye *et al.* 1992 *Revue Scientifique du Tchad*, 2 (1-2) : 83-92.
[37] Renou *et al.* 1992 *Revue Scientifique du Tchad*, 2 (1-2) : 110-112.
[38] Vaissayre *et al.* 2002. *Resistance Pest Management Newsletter*, 12, 20-22.
[39] Martin *et al.* 2000. *Pest Management Science*; 56, 549-544.
[40] Martin *et al.* 2002. *Pesticide Biochemical Physiology*; 74, 17-26.
[41] Achaleke *et al.* 2009. *Pest Management Science* 65, 1147-1154.

études de PCR quantitative réalisées en collaboration avec l'INRA d'Antibes sur des populations de *H. armigera* résistantes collectées au Bénin, au Burkina Faso et au Mali ont permis d'identifier des gènes d'oxydase surexprimés chez les individus résistants : CYP4M6, CYP4M7, CYP6AE11, CYP9A12, CYP332A1 et CYP337B1[42]. Par contre les recherches de mutations du gène *kdr* qui code pour la cible des pyréthrinoïdes, dans différentes populations africaines de *H. armigera* résistantes aux pyréthrinoïdes se sont révélées négatives (Pompon, rapport de stage 2003) ce qui suggère que le mécanisme de résistance ne serait pas du à une modification des canaux sodium cible des pyréthrinoïdes. Ce résultat confirme les résultats toxicologiques montrant l'absence de résistance croisée avec le DDT qui a la même cible que les pyréthrinoïdes.

La stratégie de gestion de résistance élaborée en Australie par Forrester *et al.* 1993) a été adaptée au parasitisme du cotonnier en Afrique de l'Ouest. Elle est basée sur l'utilisation de différentes familles insecticides dans des fenêtres successives d'une durée d'un mois réduisant ainsi la pression de sélection sur *H. armigera*. Comme nous avions montré que le spinosad, l'indoxacarb et l'endosulfan, étaient efficaces contre les chenilles de *H. armigera*, et que le mécanisme décrit comme étant à l'origine de la résistance aux pyréthrinoïdes n'avait pas été observé avec ces molécules, elles ont été recommandées pour remplacer les pyréthrinoïdes en début de campagne[43]. Pour une raison de coût l'endosulfan a d'abord été utilisé sur les deux premiers traitements. Après son interdiction d'utilisation par le CILSS (Comité Inter-Etats de Lutte contre la Sécheresse au Sahel) en 2008 en raison de sa toxicité sur l'homme et son impact environnemental il a été remplacé par d'autres molécules moins toxiques (ex : spinosad, indoxacarb, flubendiamide, spirotetramate). Nous avons montré aussi pourquoi certains organophosphorés (OPs), tel que le triazophos, étaient plus toxiques pour les individus résistants que pour les sensibles. Cet OP devant être activé par une oxydase pour être neuro-toxique nous avons montré que l'augmentation de la quantité d'oxydases chez l'insecte résistant augmentait la dégradation des pyréthrinoïdes mais accroissait aussi l'activation du triazophos[44]. D'autre part nous avons montré que certains OPs pouvaient accroître la toxicité des pyréthrinoïdes sur les chenilles résistantes en étant une cible concurrente pour les mêmes oxydases[45].

Sur la base de ces résultats, la stratégie utilisée pour gérer la résistance de *H. armigera* aux pyréthrinoïdes a été la suivante : 1/ Deux premiers traitements à l'endosulfan pour contrôler *H. armigera* sans sélectionner les individus résistants ; 2/ Suivis de deux traitements avec un mélange pyréthrinoïde plus organophosphoré synergisant pour accroître leur efficacité ; 3/ Suivis encore de deux traitements avec un mélange pyréthrinoïde plus néonicotinoide pour contrôler les aleurodes en fin de campagne. Il était nécessaire de conserver les pyréthrinoïdes au stade fructifère du cotonnier afin de pouvoir contrôler les 4 espèces de chenilles de la capsule : *H. armigera*, *Diparopsis watersi*, *Pectinophora gossypiella* et *Cryptophlebia leucotrata*.

Cette stratégie de gestion de résistance (RPM) a été diffusée en une campagne agricole auprès de plus d'1 million d'agriculteurs répartis sur 6 pays cotonniers d'Afrique de l'Ouest, grâce aux services de Recherche & Développement des sociétés cotonnières impliqués non seulement dans la collecte du coton graine, mais aussi dans l'achat et la distribution des

[42] Brun-Barale et al. 2010. *Pest Management Science* 66, 900-909.
[43] Ochou *et al.* 2001. In *Proceedings of World Conference Resistance 2001, IACR Rothamsted, UK, 24-26 September*.
[44] Martin *et al.* 2003. *Insect Biochemistry & Molecular Biology,* 33, 883-887.
[45] Martin *et al.* 2003. *Journal of Economic Entomology.*, 96, 469-474.

insecticides à crédit[46]. Son succès à l'échelle régionale a pu se mesurer par l'absence au cours des dix années suivantes de pullulation d'insecte non controlée. La réduction des traitements à base de pyréthrinoïdes, très toxiques contre les insectes prédateurs et parasitoïdes, a sans doute contribué à la réduction des pullulations d'insectes piqueurs-suceurs tels que les pucerons et les mouches blanches[47]. Cependant la mise en place de cette stratégie a entraîné une augmentation de la quantité globale d'insecticide (750 g/ha d'endosulfan ont remplacés 336 g/ha de mélange cypermethrine + chlorpyriphos) et une augmentation de 25% du coût total de la protection phytosanitaire. Aussi avons-nous poursuivi le développement des techniques permettant au planteur de n'effectuer un traitement qu'à partir d'un certain seuil d'infestation[48]. Conscients des problèmes environnementaux liés à l'utilisation de l'endosulfan, nous avons poursuivi l'évaluation des programmes de gestion de résistance avec de l'indoxacarb et du spinosad, deux nouvelles molécules plus spécifiques mais peu toxiques, pouvant être utilisées comme alternatives à l'endosulfan[49].

L'importance économique de la culture cotonnière en Afrique de l'Ouest, la diversité de son parasitisme, la rareté et le coût élevé des nouvelles molécules insecticides, la facilité avec laquelle les gènes de résistance peuvent être transférés sur de larges zones[50], le risque de résistance croisée à différentes familles insecticides quand il s'agit d'un mécanisme faisant intervenir des oxydases[51], mais aussi le risque de sélectionner de nouveaux gènes de résistance[52], remettent en cause, s'il le fallait, l'intérêt économique et environnemental du recours systématique à la lutte chimique « sur calendrier » telle qu'elle est encore appliquée par la majorité des agriculteurs en Afrique subsaharienne.

D'autres stratégies de protection du coton ont été développées ces dix dernières années : les traitements sur seuil, la lutte biologique et l'utilisation de variétés de coton génétiquement modifiées ou coton Bt (Ferron *et al.* 2006). Les interventions sur seuil permettent de réduire de 50% la quantité d'insecticide par rapport à un programme calendaire classique mais nécessitent une formation des agriculteurs à la reconnaissance des ravageurs. Le « coton biologique » interdit tout recours aux insecticides et aux fertilisants chimiques mais les surfaces restent limitées en raison des faibles rendements. Le recours à des variétés de coton génétiquement modifiées ou « coton Bt » a permis de réduire de six à deux le nombre de traitements recommandés au Burkina Faso soit une réduction de 800.000 litres d'insecticides pour une superficie emblavée de 200.000 ha en 2012. La stratégie du « coton Bt » est sans aucun doute celle qui a le plus contribué à la réduction du recours aux insecticides chimiques en Afrique subsaharienne. Mais la qualité des semences produites pose quelques problèmes en raison de la résurgence de *Dysdercus volkeri*, un ravageur secondaire qui devait auparavant être bien contrôlé par les insecticides chimiques (Hema, INERA, communication personnelle). Sur le plan de la santé publique et de l'environnement c'est un résultat positif incontestable.

[46] Martin et al. 2005. *Agriculture, Ecosystems and Environment*, 107, 409–411
[47] Ochou et Martin 2002. *Resistance Pest Management Newsletter,* 12, 10-16.
[48] Ochou *et al.* 2001. 3*me réunion du PRAO, Bobodioulasso, Burkina Faso, mars 1999* .
[49] Ochou & Martin. 2003. *World Cotton meeting, Cape Town, March 2003* .
[50] Raymond *et al.* 1991. *Nature, 350, 151.*
[51] Daborn *et al.* 2002. *Science, 297, 2253.*
[52] Gunning et al. 1991. Pestic. Sci., 33: 473–490.

Cependant *H. armigera* n'est pas le seul ravageur du cotonnier. Qu'en est-il des autres et en particulier des piqueurs-suceurs telle que l'aleurode *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) vecteur de phytovirus et responsable du « coton collant » ?

5.2 Impact de la lutte chimique sur l'aleurode *Bemisia tabaci*

En effet, l'aleurode *B. tabaci* est l'un des principaux ravageurs du coton et des cultures maraîchères dans le monde entier. Ce ravageur cause des dommages à la fois directs et indirects par leur alimentation et l'excrétion de miellat. En conséquence la stratégie de gestion de la résistance des ravageurs aux insecticides en culture cotonnière mais aussi la stratégie de protection du coton *Bt* recommandent l'application en fin de champagne de deux traitements composés généralement d'un mélange de cyperméthrine et d'acétamipride pour contrôler les chenilles et les aleurodes. Depuis les années 2000 en Afrique de l'Ouest, l'acétamipride a remplacé progressivement de diméthoate (OP) devenu inefficace pour contrôler *B. tabaci*.

A la suite d'échantillonnages de populations de *B. tabaci* sur différentes cultures au Burkina Faso, au Bénin et au Togo, nous avons montré pour la première fois en Afrique Sub-Saharienne la présence de *B. tabaci* appartenant au biotype Q (Figure 1). Les aleurodes de ce biotype ont été trouvées seules ou vivant en sympatrie avec le biotype local ASL (Afrique subsaharienne Silver Leafing)[53]. Dans le biotype Q, trois groupes ont été différenciés: Q1 pour les populations de l'Ouest Mediterranéen, Q2 pour les populations du Moyen Orient (Chu et al. 2008), et Q3, pour les populations du Burkina Faso. En effet, le biotype Q3 n'a été observé que dans les échantillons collectés au Burkina Faso. Au Bénin et au Togo, les biotypes ASL et ANSL étaient dominants dans tous les échantillons, alors qu'au Burkina Faso, c'est le biotype Q qui dominait, avec deux sous-groupes : Q1 et Q3. Le biotype Q n'a pas été observé dans les échantillons collectés dans le sud du Bénin et du Togo. Le biotype Q1 originaire de la région Méditerranéenne est généralement considéré comme un biotype invasif comme le biotype B. Il appartient bien à l'espèce *B. tabaci* (Tay *et al.* 2012) et a entamé son processus d'invasion à partir de sa région d'origine. Il a depuis été recensé dans 10 pays différents (De Barro *et al.* 2011; Dalton 2006). Les échantillons d'aleurodes n'appartenant qu'au seul biotype Q1 n'ont été trouvés que sur coton. Les biotypes ASL et Q1 ont été trouvés en sympatrie dans des échantillons collectés sur coton, tomate et gombo.

Nous avons montré que les populations de *B. tabaci* collectées au Burkina Faso présentaient généralement une résistance plus élevée aux pyréthrinoïdes, aux OPs et aux néonicotinoïdes que les populations collectées au Bénin et au Togo. Au Burkina Faso, en effet, les populations de *B. tabaci* collectées dans les champs de coton se sont avérées les plus résistantes aux pyréthrinoïdes tels que la deltamethrine et la bifenthrine, aux OPs, tels que le dimethoate et le chlorpyriphos ethyle, au carbosulfan, un carbamate et aux neonicotinoids, tels que l'acetamipride et le thiamethoxam. Les facteurs de résistance (FR) mesurés par rapport à la population de terrain la plus sensible varient en fonction de la date et du lieu de collecte et du biotype. Pour les néonicotinoïdes le FR maxi est passé en l'espace de deux ans de 8 à 50 dans les populations de

[53] Gnankiné et al. 2012 *Insect Conservation and Diversity*.

B. tabaci collectées au Burkina Faso. L'augmentation du niveau de résistance aux néonicotinoïdes peut s'expliquer par son utilisation sur coton contrairement au carbosulfan pour lequel d'ailleurs le niveau de résistance est faible.

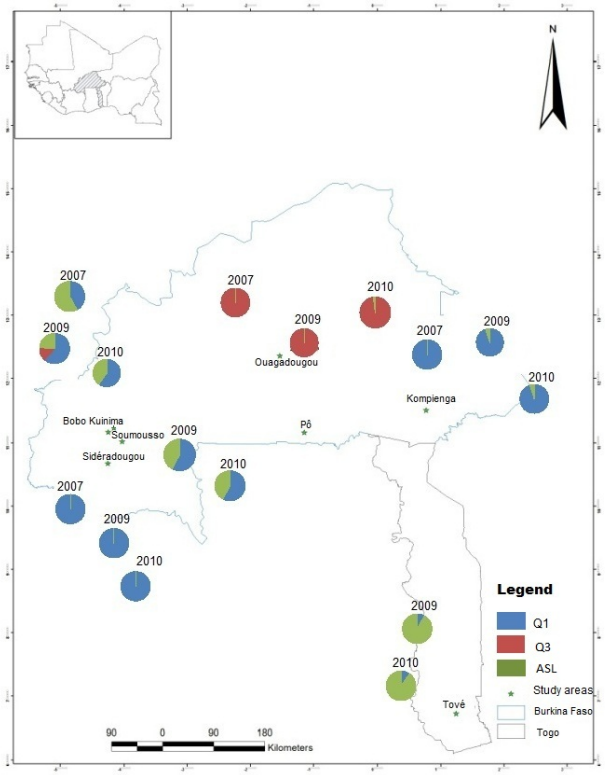

Figure 1: Dynamic of MED of *B. tabaci* species complex in Burkina Faso and Togo (2007, 2009 and 2010)[54]

Les études de cinétique enzymatique réalisées en mesurant les vitesses d'inhibition de l'acétylcholinestérase (AChE) par le composé organophosphoré chlorpyrifos-oxon nous ont permis d'estimer le nombre de génotypes possibles des populations de terrain résistantes aux OPs en les comparant à différentes souches de référence sensibles ou résistantes[55]. Dans les populations de terrain, deux allèles du gène *Ace1* ont été détectés: un allèle *Ace1-S* sensible et un allèle résistant *Ace1-R*. Les populations du Bénin contenaient clairement trois génotypes différents confirmant la présence de populations hétérogènes pas complètement résistantes. Dans une autre étude nous avons recherché la présence de mutations responsables de la résistance aux pyréthrinoïdes (gène *kdr*) et aux OP/CX (gène *Ace1-R*) dans les populations collectées en Afrique de l'Ouest. La mutation L925I du gène *kdr* qui code pour les canaux sodium, cible des pyréthrinoïdes, n'a été observée que chez les individus appartenant au biotype Q1 (Gnankiné, communication personnelle). La mutation F331W du gène *Ace1-R* qui code pour l'acétylcholinestérase, cible des OPs et carbamates, a été observée chez des individus appartenant aux biotypes ASL et Q1 mais pas au biotype Q3. La fréquence de la mutation *Ace1-R* serait très élevée (0,9 – 1) dans les biotypes ASL et Q1 collectés dans le nord du Bénin et au Burkina Faso, aussi bien sur coton que sur tomate (Gnankiné, comm. person.). Ces résultats expliquent l'inefficacité des OPs pour lutter contre *B. tabaci*.

[54] Gnankine et al 2013 International Journal of Tropical Insect Science
[55] Houndete at al 2010. *Pesticide Biochemistry and Physiology*, 98, 115-120.

Nos résultats montrent que la résistance de *B. tabaci* aux néonicotinoïdes est liée au biotype Q1. En effet, les plus forts facteurs de résistance à l'acétamipride et au thiamethoxam ont été enregistrés dans les populations de Q1 pure. Par ailleurs, les populations de *B. tabaci* les plus sensibles étaient composées d'individus appartenant aux biotypes Q1 et ASL. Au Bénin, les populations composées du biotype ASL pur étaient plus sensibles aux néonicotinoïdes que les populations hétérogènes du Burkina Faso[56,57]. Cependant la sensibilité aux néonicotinoïdes de certaines populations de *B. tabaci* du biotype Q1 montre qu'il y a toujours des individus sensibles aux neonicotinoïdes même si cela n'exclut pas la résistance à d'autres insecticides (pyréthrinoïdes et OPs). Nos résultats ont montré aussi une résistance croisée entre les néonicotinoïdes. La population de Sideradougou par exemple, était à la fois très résistante à l'acétamipride (23,8 fois) et au thiametoxam (24,7 fois). La résistance de *B. tabaci* aux néonicotinoïdes serait due à une augmentation de l'activité métabolique des oxydases (Rauch et Nauen 2003). En effet Karunker *et al.* (2009) ont montré que la surexpression du gène CYP6CM1 était associée à des niveaux élevés de résistance de *B. tabaci* à l'imidaclopride.

La présence du biotype invasif Q1 de *B. tabaci* et sa résistance aux néonicotinoïdes en Afrique Sub-saharienne est plutôt préoccupante. En effet, à moins de supprimer l'usage des néonicotinoïdes sur coton et cultures maraîchères, ce biotype Q1 devrait progressivement remplacer le biotype local ASL. Cela signifie qu'à court terme les néonicotinoïdes ne seront plus efficace pour contrôler ce ravageur au même titre que les pyréthrinoïdes et les OPs. Pour la production cotonnière cela peut entrainer une augmentation du taux de collage de la fibre de coton, due aux déjections de *B. tabaci* sur les capsules ouvertes en fin de campagne. Cependant ce problème peut toujours être évité par une récolte précoce. Par contre l'enjeu est bien plus grave pour les cultures maraîchères cultivées principalement en saison sèche. D'une part parce que les pullulations de *B. tabaci* ont un impact direct sur le développement des jeunes plantes et que d'autre part parce que la propagation d'un vecteur de bégomovirus tels que le TYLCV, par le biotype invasif qui pourrait être plus prolifique que le biotype local, pourrait menacer la production de solanacées cultivées généralement en plein champs par des petits producteurs (Hanafi, 2000). Cette sélection d'un biotype Q1 multi-résistant paraît inévitable car les traitements aleurodicides contre *B. tabaci* en culture cotonnière sont surtout un argument commercial pour justifier l'absence de « coton collant » dans leur production et d'éviter ainsi une décote globale de toute la production. Et qu'une partie de ces insecticides coton est ensuite inévitablement transférée sur les cultures maraîchères.

Nos résultats montrent aussi que *B. tabaci* est un bon indicateur de la pression insecticide. En effet *B. tabaci* est un ravageur du coton et des cultures maraîchères. Il est facile à trouver toute l'année et en quantité suffisante. Il est résistant aux trois grandes familles d'insecticide : pyréthrinoïdes, OPs/carbamate et néonicotinoïdes. Par ailleurs les principaux gènes de résistance sont maintenant identifiés (*kdr*, *Ace-1* et *Cyp6CM1*). Il est donc devenu possible de suivre avec précision la fréquence des gènes de résistance dans les populations échantillonnées.

[56] Houndete et al. 2010. *Pest Management Science* 66: 1181–1185.
[57] Gnankine et al. 2012. *Insect Conservation and Diversity*.

5.3 Impact de la lutte chimique sur le vecteur du paludisme *Anopheles gambiae*

En terme d'effet indirect de la lutte chimique sur la santé publique je me suis intéressé à l'impact des traitements agricoles sur la résistance aux insecticides du moustique *Anopheles gambiae* Giles (Diptera: Culicidae), principal vecteur du paludisme en Afrique subsaharienne. Il se trouve en effet que la plupart des méthodes de lutte recommandées par l'OMS pour protéger les populations contre le paludisme reposent sur la lutte chimique à travers l'utilisation de moustiquaires imprégnées de pyréthrinoïdes à longue durée d'action (MII) et de traitements intra-domiciliaires avec des OPs, voire également le traitement des gites larvaires. La sélection des populations d'*An. gambiae* résistantes à ces insecticides est donc préoccupante car elle menace l'efficacité de ces méthodes de lutte contre le paludisme et qu'il n'existe pas à l'heure actuelle d'alternative à ces deux familles chimiques.

Les entomologistes médicaux spécialistes du control des vecteurs du paludisme soupçonnent depuis longtemps les traitements agricoles d'être responsables de la sélection des moustiques aux insecticides. Avec les entomologistes médicaux et agricoles travaillant au Bénin, au Burkina et au Togo nous avons mis en place un réseau « santé/agriculture » pour étudier ce phénomène. Ce réseau financé à travers le projet CORUS 6015 de l'AIRD a été réalisé en collaboration avec le Cirad (PI-Nord), l'IRD-UMR MIVEGEC, le CREC (PI-Sud) et l'INRAB du Bénin, l'IRSS et l'Université de Ouagadougou au Bukina Faso, l'Université de Lomé au Togo et les Universités de Toulouse et de Lyon. Ce projet a permis de contribuer au financement des thèses de Thomas Houndete et Anges Yadouleton au Bénin et de Moussa Namountougou au Burkina Faso. Il a permis également d'établir une collaboration avec l'université de Lyon avec l'accueil en post doctorat du Dr Olivier Gnankiné de l'université de Ouagadougou.

La sélection de la résistance aux pyréthrinoïdes et aux OPs chez les populations de vecteurs du paludisme comme *An. gambiae* serait en grande partie liée à l'utilisation actuelle de ces mêmes insecticides en agriculture. Entrainés par le vent et les pluies, les résidus contaminent les gîtes de reproduction des moustiques où sont sélectionnées les larves résistantes tout au long de leur développement larvaire. À ce jour, la lutte anti-vectorielle a principalement portée sur les moustiques adultes par la pulvérisation intra-domiciliaire et l'utilisation de moustiquaires imprégnées d'insecticide à longue durée d'action (Kelly-Hope *et al.* 2008 ; Raghavendra *et al.* 2011). Or la résistance d'*An. gambiae* aux pyréthrinoïdes réduirait déjà l'efficacité des moustiquaires imprégnées (N'Guessan *et al.* 2007).

An. gambiae s.s. contient deux formes moléculaires (M et S) qui co-existent en Afrique occidentale (Della-torre *et al.* 2005). Dans les savanes sèches, la forme M est prédominante dans les sites de reproduction permanents tels que les mares ou les rizières, alors que la forme S est prédominante dans les habitats temporaires ensoleillés, notamment les flaques d'eau produites par les pluies pendant les saisons humides. Les champs désherbés et le bord des chemins d'accès sont donc des zones de prédilection pour les pontes d'Anopheles en saison des pluies.

Au Bénin et au Burkina Faso, les bio-essais réalisés avec les tests diagnostiques de l'OMS ont montré une fréquence élevée de la résistance à la perméthrine, au bendiocarbe, au

fénitrothion et au DDT chez les formes M et S d'*An. gambiae* S.S.[58,59,60]. Parmi les OPs et les carbamates testés, seul le bendiocarbe a été recommandé en traitement intra-domiciliaire pour compléter l'efficacité des moustiquaires imprégnées.

La fréquence des gènes conférant une résistance aux insecticides est très différente chez les formes M et S et aussi chez *An. arabiensis*[61]. La résistance d'*An. gambiae* au DDT et aux pyréthrinoïdes est conférée en particulier en Afrique occidentale par la mutation *kdr* L1014F qui code pour le canal sodium cible des deux familles chimiques. Cette mutation n'a d'abord été trouvée que dans les populations d'*An. gambiae* forme S. Puis cette mutation a sans doute été sélectionnée par les traitements à base de pyréthrinoïdes dans les agro-systèmes cotonniers où sont réunies les conditions favorables aux gîtes larvaires à savoir des petites flaques d'eau temporaires et ensoleillées. Cette mutation a ensuite été retrouvée chez des individus de forme M soit qu'elle a été sélectionnée soit qu'elle a été transférée par introgression (Diabate *et al.* 2003). Il faut préciser qu'en Afrique subsaharienne les traitements insecticides sont plutôt rares en riziculture, comme sur la plupart des céréales. Aujourd'hui, les deux mutations L1014F, L1014S coexistent et sont largement distribués dans toute l'Afrique subsaharienne. Par ailleurs, une acétylcholinestérase insensible (mutation *Ace1-1R*) est impliquée dans la résistance aux organophosphorés et carbamates (OP / CX) dans les populations d'*An. gambiae* d'Afrique de l'Ouest. Cette mutation est une seule substitution d'une glycine en sérine en position 119, du gène qui code le site catalytique AChE1 (G119S) (Weill *et al.* 2004). Cette mutation a été récemment observée chez quelques *An. gambiae* collectés au Bénin et dans l'ouest du Burkina Faso (Djogbenou *et al.* 2008, 2010). La résistance d'*An. gambiae* aux insecticides peut aussi être due à une modification de l'activité enzymatique. Au Bénin, nos études ont montré une activité plus élevée des monooxygénase (P450), estérase ou glutathion-S-transférase (GST) dans les populations de terrain résistantes au DDT, à la perméthrine et au carbosulfan par rapport à la souche de référence Kisumu[62]. Au Burkina Faso, un niveau plus élevé d'estérase et de GST a été observé chez les deux formes moléculaires d'*An. gambiae* comparé à la souche Kisumu. Ces résultats sont à prendre avec précaution car ils peuvent être dus aux différences de pression environnementale entre les souches de terrain et la souche Kisumu élevée au laboratoire depuis plus de 30 ans. Cependant Muller *et al.* (2008) ont identifié récemment un gène de P450, CYP6P3, surexprimé chez les individus résistants aux pyréthrinoïdes d'une population sauvage d'*An. gambiae* collectée au Ghana.

Les fréquences des mutations des gènes *kdr* et *Ace-1R* ont été représentées sur une carte en rapport avec les formes moléculaires illustrant la situation de la résistance d'*An. gambiae* au Burkina Faso (Figure 2). Les fréquences de mutation du gène *kdr* les plus élevées ont été observées aussi bien dans les anciennes que dans les nouvelles régions cotonnières du Burkina Faso[63]. La mutation *kdr* est beaucoup plus fréquente chez *An. gambiae* forme S que chez la forme sympatrique M. Elle a aussi été récemment retrouvée chez quelques individus de l'espèce *An.*

[58] Corbel et al 2007. Acta Tropica,101, 207-216.
[59] Djegbe et al 2011. *Malaria J*, 10: 261.
[60] Dabire et al. 2012. In *Insecticides—Pest Engineering*.
[61] Dabire et al. 2012. In *Insecticides—Pest Engineering*.
[62] Corbel et al, 2007. *Acta tropica*, 101, 207-216.
[63] Namountougou et al 2010. *Acta Tropica*

arabiensis. *An. gambiae* forme S est prédominant dans l'ouest, qui est aussi la zone cotonnière la plus ancienne, sauf dans les localités VK5 et VK7 proches des zones rizicoles. La forme S a également été observée dans la majorité des régions de l'Est. Dans la partie centrale du Burkina Faso, les formes M ont été trouvées avec une prévalence modérée. Dans les zones où les deux formes sont présentes, la fréquence de mutation est plus élevée en fin de saison des pluies, avec un pic maximum en octobre. La mutation *Ace-1R* est bien moins répandue que la mutation *kdr* chez *An. gambiae* S.S. Elle a été trouvée chez moins de 1% d'*An. gambiae* collectés principalement dans les zones cotonnières. Cela peut s'expliquer par le coût pour les insectes de la résistance aux OPs/CX due à une acétylcholinestérase modifiée qui leur donne un handicap en l'absence d'insecticide contrairement à la mutation *kdr*.

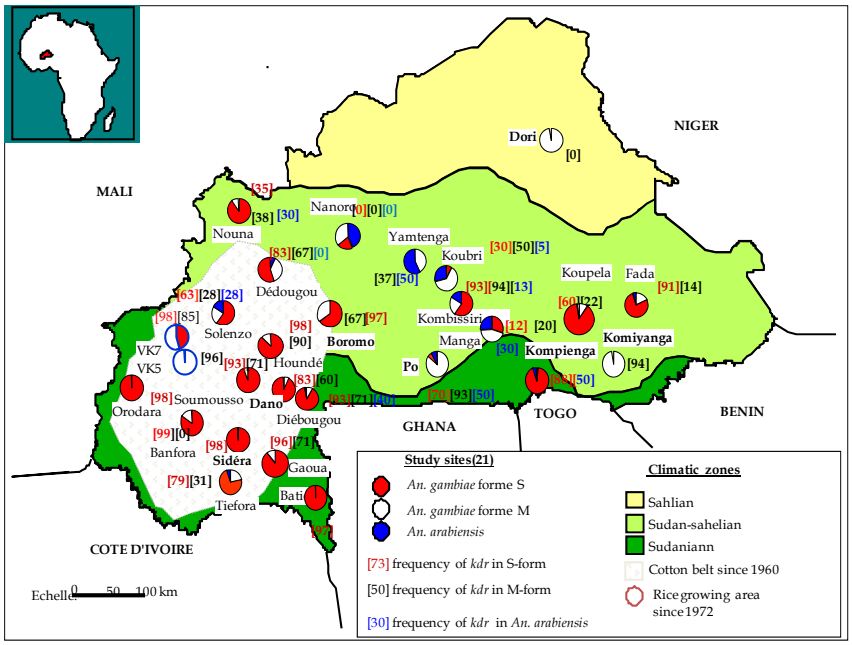

Figure 2 : Geographic distribution of L1014F kdr allele in An. gambiae s.l. populations including pyrethroids and DDT resistance profile in Burkina Faso in 2009 (numbers in braket represent frequency of L1014F kdr allele frequencies) [64]

Les études réalisées ces dix dernières années montrent une diffusion de la résistance aux pyréthrinoïdes (et dans une moindre mesure aux OPs/CX) dans toutes les sous populations d'*An. gambiae* à partir de la population S que l'on retrouve principalement dans les zones cotonnières et pour laquelle cette résistance est quasi fixée. On observe également une addition des mécanismes de résistance dus à une modification de la cible (*kdr*, *ace-1*) ou à une augmentation du métabolisme (CYP6P3, estérase ?, GST ?). Les *An. gambiae* collectés dans les zones de culture cotonnière et maraîchère présentent systématiquement les facteurs de résistance et les fréquences de gène *kdr* les plus élevés par rapport aux populations collectées dans les zones de culture peu ou pas traitées (Djogbenou comm. perso.). Par ailleurs une étude effectuée sur des populations d'*An. gambiae* collectées dans différentes zones cotonnières a montré que la fréquence de résistance *kdr* augmentait en fonction du nombre de traitements (T) réalisés sur coton : coton biologique (0 T), traitements sur seuils (1-3 T), traitements calendaires (4-6 T)[65].

[64] Dabire et al, 2012. In Pest Engineering
[65] Yadouleton et al. 2011. *Parasites & Vectors,* **4,** 60

La réduction des traitements insecticide sur les variétés de coton génétiquement modifiées au Burkina Faso devrait avoir un impact la résistance des *An. gambiae* aux insecticides du fait d'une réduction de la pression de sélection. Le niveau de résistance aux pyréthrinoïdes devrait se stabiliser et la nouvelle mutation L1014S ne devrait pas être sélectionnée. La résistance aux OPs quant à elle devrait disparaître en raison de son coût.

5.4 Pratiques phytosanitaires en culture maraîchère

En Afrique subsaharienne les villes grossissent et se multiplient. L'influence des marchés urbains sur les productions rurales s'accentue, les populations sont de plus en plus mobiles et les régimes alimentaires se modifient (Mougeot et Moustier, 2009). Divers facteurs poussent les productions agricoles urbaines à accroître leur part dans l'approvisionnement alimentaire des villes de cette région. Depuis une dizaine d'années, l'agriculture urbaine est reconnue comme un enjeu majeur en termes d'approvisionnement des villes, d'emploi et de gestion de l'environnement urbain (Undp, 1996). Cependant, les outils de diagnostic des problèmes de cette agriculture et les moyens d'intervention pour son développement durable font défaut aux chercheurs, décideurs et agents du développement, traditionnellement tournés vers les zones rurales, et aux spécialistes de la gestion urbaine, peu familiers du monde agricole.

La protection des cultures de légumes contre les ravageurs est une composante essentielle de la production maraîchère en zone tropicale. Il s'agit d'une part de cultures « exotiques » telles que la tomate, la salade, le chou, la carotte, le concombre, l'aubergine et le haricot vert principalement en zone urbaine, et d'autre part d'espèces locales comme l'aubergine africaine, le piment, la morelle, l'amaranthe, et d'autres légumes feuilles « indigènes» toujours très appréciés par les populations urbaines. Il faut avoir en tête que la pression parasitaire est extrêmement forte et variée toute l'année car il n'y a pas de période froide pour réduire les populations d'insectes. Et plus particulièrement pendant la saison sèche car la réduction des ressources alimentaires rend les cultures irriguées encore plus attractives.

Avec Claude Ahouangninou que j'encadre en thèse de doctorat sous la direction du Pr Fayomi, médecin du travail et doyen de l'Université d'Abomey Calavi (UAC) nous avons cherché à caractériser les pratiques phytosanitaires des maraîchers de Tori-Bossito, une commune voisine de Cotonou, capitale économique du Bénin[66]. Par la suite nous avons complété cette étude en utilisant des indicateurs pour évaluer les risques sur la santé humaine et l'environnement liés aux pesticides utilisés[67]. Enfin nous avons élaboré une méthode biologique pour évaluer la teneur en résidus d'insecticide dans des échantillons de plante et de sol.[68]

En 2008 à Tori-Bossito, les maraîchers exploitent des superficies allant de 0,02 à 3 ha. La production de légumes est destinée à la commercialisation sur les marchés de Cotonou. Les 108 agriculteurs enquêtés utilisent régulièrement des pesticides : 100% des insecticides et 68% des fongicides. Les formulations insecticides sont à plus de 90% des mélanges de pyréthrinoïde et d'OP ou de l'endosulfan complété par des pyréthrinoïdes seuls (cyperméthrine ou deltaméthrine)

[66] Ahouangninou et al. 2011. *Cahiers de l'Agriculture*, 20, 3, 216-222.
[67] Ahouangninou et al. 2012. *Journal of Environmental Protection*, 3, 241-248.
[68] Ahouangninou et al. 2013. *British Biotechnology Journal*

ou de l'endosulfan seul. Dans cette commune, 84% des maraichers utilisent du COTALM P 218, une formulation destinée à la protection du coton composée de lambdacyhalothrine et de profenofos (18+200 g/l). Les principaux fongicides utilisés sont le mancozèbe ou le méthylthiophanate. Seuls 3% des maraîchers utilisent du carbofuran contre les nématodes. La majorité des producteurs (62 %) ont acheté ces formulations reconditionnées dans des petites bouteilles auprès de petits détaillants, tandis que 38 % ont acheté leurs pesticides conditionnés dans les flacons d'origine chez des grossistes. Pour prévenir les attaques de ravageurs, la majorité des producteurs font régulièrement un traitement de toute leur parcelle, quelque soient les cultures et leur stade de développement. Au cours de notre enquête environ 70 % des producteurs auraient effectués 4 à 5 traitements insecticides par mois, mais 20 % d'entre eux en auraient fait 6 à 7 sur les cultures de haricot vert, de concombre et d'aubergine. Moins de 7 % des producteurs n'en auraient fait que 2. D'après nos propres observations, les producteurs n'appliquent généralement pas les doses recommandées sur les emballages. Soit parce qu'il n'y en a pas, soit parce qu'il ne savent pas lire (57% sont analphabètes). Pour mesurer la quantité de produit à mélanger à l'eau dans l'appareil de traitement, les maraîchers utilisent le bouchon de la bouteille, ou de petits verres, ou d'autres petits contenants. Les dosages appliqués lors d'un traitement que nous avons calculé en tenant compte de la concentration de la formulation, du volume utilisé et de la surface traitée ont varié de 410 à 720 g de matière active par hectare (m.a/ha) avec une moyenne de 510 g m.a/ha contre 218 g m.a/ha recommandé pour le COTALM P 218EC en culture cotonnière. En plus de cela les délais de traitement avant récolte ne sont généralement pas respectés : 70% des producteurs appliquent des délais de 3 à 5 jours et 10% un délais de 1 à 2 jours. Si 70% des maraîchers utilisent des pulvérisateurs à dos à pression entretenue, 30% utilisent des branchages pour asperger leurs cultures. Lors de la préparation du mélange insecticide comme de son application, la majorité des maraîchers sont pieds et torse nu avec un short ou un pantalon. Seuls 4% des maraîchers enquêtés affirmaient porter une tenue de protection. Pourtant tous les producteurs reconnaissent les dangers de pesticides. Ils citent des irritations de la peau (39%), des rhumes (37%), des céphalées (16%), des bouffées de chaleur (8%) des vertiges (7%). Aussi la majorité des maraîchers se lave après la réalisation d'un traitement. Mais si la moitié des maraîchers gardent les pesticides au champ, dans les buissons ou enterrés, l'autre moitié les stocke à la maison dans une pièce à part (13 %) ou dans leur chambre (41 %), constituant ainsi un risque pour la santé de toute la famille. Quant aux modes de gestion des emballages vides, 46 % des maraîchers les réutilisent pour se procurer de nouveaux pesticides chez le détaillant, 24 % les abandonnent dans les champs, 10 % les jettent dans les mares.

Malheureusement les résultats de cette enquête ne font que confirmer ceux obtenus dans d'autres pays d'Afrique subsaharienne (Cissé *et al.* 2003 ; Ngowi *et al.* 2007 ; Obopile *et al.* 2008 ; Williamson *et al*. 2008 ; Kanda *et al.* 2009 ; Abdulkadir *et al.* 2012). Pour des raisons essentiellement de coût et de facilité d'utilisation, mais aussi de manque d'information ou d'alternative efficace et disponible, le recours aux pesticides chimiques est systématique pour optimiser et sécuriser les rendements. Les agriculteurs, principalement en raison de leur faible niveau d'instruction, connaissent mal la toxicité réelle des pesticides utilisés et leur mode d'utilisation. Ils ne disposent pas de fiches techniques faisant la relation entre le ravageur, ses dégâts, le produit à utiliser, sa dose et sa fréquence, le débit de l'appareil et la surface à traiter.

L'information écrite sur les bouteilles, le plus souvent en langues étrangères (français, anglais, chinois) et les pictogrammes aux normes internationales sont mal compris (Tourneux, 1993). Du Bénin au Sénégal, les petits maraîchers ne suivent donc pas les recommandations liées à une bonne utilisation des pesticides qui ne sont pas toujours employés à bon escient et avec le matériel adéquat. D'autant plus que la production maraîchère est en majeure partie destinée au marché local où la qualité n'est pas rémunérée et où aucun control qualité n'est pratiqué : ni sur les pesticides, ni sur les pratiques phytosanitaires, ni sur la présence de résidus dans les fruits, les légumes, le sol ou les eaux de surface. Les rares études réalisées dans ce domaine montrent pourtant des risques d'intoxication humaine et de contamination du milieu pouvant être parfois très élevés (Cissé *et al.* 2003 ; Sanborn *et al.* 2004 ; Pazou *et al.* 2006a ; Pazou *et al.* 2006b ; Traoré *et al.* 2006 ; Assogba- Komlan *et al.* 2007). Les analyses chimiques sont en effet très coûteuses et nécessitent des laboratoires bien équipés quasi-inexistants en Afrique sub-saharienne. Aussi nous avons mis au point un test biologique basé sur l'utilisation de larves de moustique de l'espèce *Aedes aegypti*, afin de pouvoir évaluer le risque de présence de résidus d'insecticide dans les fruits et légumes[69]. Ce test développé initialement pour doser les pyréthrinoïdes dans les moustiquaires[70] nécessite de l'éthanol, des œufs d'Aedes et de l'eau. Il est simple, reproductible et facile à utiliser. Il suffit d'ajouter un extrait alcoolique d'échantillon de plante ou de sol dans un gobelet d'eau contenant des larves d'*Ae. aegypti* fraichement écloses et de regarder la mortalité à 24h : les larves mortes flottent à la surface. L'intérêt d'utiliser des larves d'*Ae. aegypti* est leur forte sensibilité aux pyréthrinoïdes (LC_{50}=0.4 ppb). Il est très facile de se procurer des œufs dans les Centre de démoustication comme le CREC de Cotonou, Benin et ces œufs peuvent être conservés plusieurs mois à température ambiante.

Cette technique a été utilisée pour évaluer le risque de teneur en résidus de pesticide sur des échantillons de 8 légumes feuille « indigènes » prélevés à la récolte sur plusieurs sites maraîchers autour de Cotonou[71]. En moyenne, 30% des échantillons se sont révélés positifs avec des teneurs de 5 à 100 ppb en équivalent deltaméthrine. Les plus fortes teneurs résiduelles ont été retrouvées dans des échantillons de *Solanum macrocarpum* (gboma) qui subit actuellement de fortes attaques de l'acarien invasif *Tetranychus evansi* et que les maraîchers n'arrivent pas à contrôler… Cette technique a également été utilisée sur des échantillons de 14 légumes prélevés sur les marchés de Cotonou. 12,5% des échantillons se sont révélés positifs avec des teneurs moyennes de 2 à 9 ppb d'équivalent deltaméthrine retrouvés dans des laitues, des tomates, du piment, et du basilique.

5.5 L'impact des traitements agricoles sur la santé et l'environnement

La lutte chimique est pratiquée en zone cotonnière sur plus d'1 M d'hectares en Afrique de l'Ouest et du Centre. Même si le nombre de traitement reste faible par rapport à d'autres régions cotonnières comme en Inde (Kranthi *et al.* 2002), 4 traitements en moyenne réalisés à 14 jours d'intervalle pendant les 5 mois de saison des pluies représentent néanmoins chaque année un volume de 4 M de litres d'insecticide. En comparaison les quantités de pesticides appliquées sur

[69] Ahouangninou et al. 2013. *British Biotechnology Journal* (accepté)
[70] Martin et al. 2007. *Tropical Medicine and International Health*, 12: 245-250.
[71] Ahouangninou et al. 2013. *British Biotechnology Journal* (accepté)

les cultures maraîchères toute l'année à des doses et des fréquences beaucoup plus élevées sont difficiles à évaluer, d'autant qu'il n'y a pas vraiment de filière organisée et que les superficies sont en constante augmentation du fait de la croissance démographique et urbaine. Si sur les 6 traitements insecticides fournis à crédit par les sociétés cotonnières aux producteurs de coton, 2 traitements sont détournés sur les cultures maraîchères, cela ferait environ 2 M de litres d'insecticide. Quoiqu'il en soit la concentration de la pollution chimique qui en découle dans et autour des centres urbains en particulier doit avoir un impact direct non négligeable sur la santé des populations mais aussi de façon indirecte par la sélection d'insectes vecteurs de maladies et multi-résistants aux insecticides recommandés par l'OMS.

En culture cotonnière, les stratégies de traitement sur seuil permettent de réduire de moitié la quantité d'insecticide utilisée par rapport aux traitements calendaires. Cette stratégie se diffuse au coup par coup car elle nécessite une formation de base et continue en raison de l'évolution du parasitisme. L'utilisation des variétés de coton génétiquement modifiées ou « coton bt » a permis de réduire encore plus le recours à la lutte chimique (de 0 à 2 traitements). Cette stratégie a pu être développée rapidement sur de grandes superficies au Burkina Faso même si de nouveaux problèmes sont apparus comme la réduction de la production de graines due aux dégâts de la punaise *Dysdercus voelkery*. Ces stratégies contribuant à la réduction voire à la suppression de la lutte chimique permettent dorénavant le développement d'autres méthodes de lutte plus respectueuses de l'environnement : lâchers de prédateurs et de parasitoïdes, pulvérisation d'entomopathogènes, cultures associées (push-pull), pièges attractifs, confusion sexuelle…

Par contre en cultures maraîchères la pression insecticide est continuelle et croissante du fait du faible coût des pesticides sur le marché local, de la sélection d'insectes ravageurs multi-résistants, de la forte valeur ajoutée de la production, de l'augmentation de la demande et donc de l'extension des surfaces pour alimenter les centres urbains etc. Cette situation rend extrêmement difficile toute stratégie de protection dite « biologique » voire « raisonnée ». A titre d'exemple, un recensement des acariens dans les périmètres maraîchers du Sud Bénin a montré la présence de 3 espèces d'acariens phytophages mais aucun acarien prédateur n'y a été observé (Adango *et al.* 2006). En comparaison, plus d'une dizaine d'espèces prédatrices ont été identifiées sur les parcelles isolées et non traitées de la station de recherche de l'IITA à Abomey Calavi.

Pourtant les consommateurs urbains se préoccupent de plus en plus de la qualité de leur alimentation suite aux cas d'intoxication dus à une mauvaise utilisation des pesticides. La création d'un réseau AMAP à Cotonou en 2006, basé sur la communauté expatriée a permis à un agriculteur béninois, Edgar Deguegnon, de produire mieux (et moins) mais en vendant sa production à un prix supérieur au marché local. Mais cela reste un cas exceptionnel. Actuellement la qualité ne se vend pas mieux. Il faut donc pouvoir produire mieux tout en produisant autant. Parallèlement à cela depuis le début du millénaire, les bailleurs de fonds (Roll Back Malaria, Gates Fondation, WHO, Unicef…) ont largement contribué à diffuser l'usage des moustiquaires pour lutter contre le paludisme. De nouvelles industries se sont donc développées en Afrique subsaharienne (Vestergard Frandsen, A to Z Textile Mills) pour produire ces moustiquaires en grande quantité et les rendre disponibles sur le marché local. Il ne restait plus

qu'à transférer et adapter cette technologie à la protection des cultures maraîchères avant que les maraîchers n'utilisent leur propre moustiquaire de lit.

5.6 Des filets anti-insecte pour réduire les insecticides en cultures maraîchères

5.6.1 Protection contre les insectes

La culture de chou est de loin la plus traitée par les insecticides chimiques en Afrique subsaharienne (de 1 à 2 traitements par semaine pendant les 3 mois de culture). Cela s'explique par la capacité de la teigne du chou, *Plutella xylostella*, à résister rapidement à toutes les familles chimiques. A tel point qu'en 2004 beaucoup de maraîchers au Bénin avaient abandonné sa production pourtant très prisée surtout en période de fête. Les filets en polyester de type moustiquaire (maille de 2 mm) que nous avons testé se sont vite révélés extrêmement efficaces pour protéger les cultures de chou non seulement au stade pépinière avec une couverture permanente mais aussi et surtout après repiquage des plants[72]. Les filets se sont aussi révélés efficaces contre un autre lépidoptère, *Hellula undalis*, bien plus dangereux que *P. xylostella*, car une chenille d'Hellula suffit à détruire un plant à elle toute seule. Cependant ce ravageur est peu connu des maraîchers car il est facilement contrôlé par les insecticides. Par contre les noctuelles de *Spodoptera littoralis*, ravageurs occasionnels, peuvent pondre des zooplaques sur les filets et c'est alors des dizaines de larves qui descendent sur les choux à l'aide d'un fil de soie. Ces insectes sont également sensibles aux insecticides et un traitement insecticide suffit à les éliminer.

En raison de la taille des mailles utilisées les filets ne permettent pas de protéger les choux contre les pucerons tels que *Myzus persicae* et *Lipaphis erysimi* même si la barrière visuelle permet parfois de retarder les infestations[73]. Dés qu'ils passent le filet les pucerons peuvent très vite proliférer en toute quiétude. En effet, les filets sont aussi une barrière pour les prédateurs et les parasitoïdes même si ces derniers peuvent passer au travers. Nous avons montré que plus les mailles sont petites, plus le taux de parasitisme des pucerons est faible. Une solution consiste donc à ouvrir les filets pendant la journée (1 à 2 fois par semaine). Cela permet aux insectes utiles de visiter la culture et de parasiter les ravageurs présents. Cela permet aussi à l'agriculteur de vérifier la bonne santé des plantes et l'absence de chenilles telles que *S. littoralis*.

Malgré tout, les filets ont permis de réduire de 70% à 100% le nombre de traitements insecticides des maraîchers par rapport à leur pratique habituelle. Après le succès des expérimentations et des démonstrations de la technique auprès des petits maraîchers au Bénin[74] et au Kenya[75,76], les filets anti-insectes destinés au petits maraîchers sont maintenant en phase de diffusion. Notre partenaire industriel dans les projets BioActiveNet et BioNetAgro, la société tanzanienne A to Z Textiles Mills qui fabrique plus de 30 M de moustiquaires imprégnées par an, devrait produire et diffuser cette année au Kenya le filet « cabbage net 1.0 ». Deux doctorants en économie, Patrick Muthee au Kenya (Cirad, Egerton University & CNRS-Crdi Université

[72] Martin et al 2006. Journal of Economic Entomology
[73] Martin et al 2013. Journal of Economic Entomology
[74] Licciardi et al 2008. Journal of Tropical Insect Science
[75] Gogo et al 2012. *Hort Technology*, 22, 292-298
[76] Muleleke et al 2013. *Agronomy* 3, 2.

d'Auvergne) et Faustin Vidogbena au Bénin (Cirad, Université d'Abomey Calavi), font actuellement leur thèse de doctorat sur les conditions d'adoptions de cette technologie par les petits agriculteurs.

En parallèle nous avons étudié l'efficacité de filets imprégnés de pyréthrinoïde à longue durée d'action pour améliorer l'efficacité de ces filets anti-insecte contre les pucerons et réduire les risques de ponte des papillons de *S. littoralis*. L'hypothèse est qu'un filet répulsif pourrait permettre d'éviter tout recours aux insecticides chimiques. Cette technique est recommandée depuis plusieurs années par l'OMS et diffusée largement en Afrique subsaharienne pour protéger les populations contre le vecteur du paludisme *An. gambiae*. Nous n'avons fait que l'adapter à la protection des plantes contre les insectes piqueurs (pucerons, aleurodes) en utilisant des filets à maille plus petite (0,9 mm au lieu de 2 mm). Au laboratoire nous avons montré l'effet irritant et répulsif de ces filets sur l'aleurode *Bemisia tabaci*, les pucerons *Myzus persicae* et *Lipaphis erysimi* et les papillons de la teigne du chou *P. xylostella*. Les filets imprégnés d'alphacyperméthrine réduisent significativement le taux de passage des pucerons et des mouches blanches par rapport à un filet non traités. A Montpellier en France, leur efficacité a même été totale au champ pour protéger des plants de choux contre les pucerons montrant par là la preuve du concept (Figure 3).

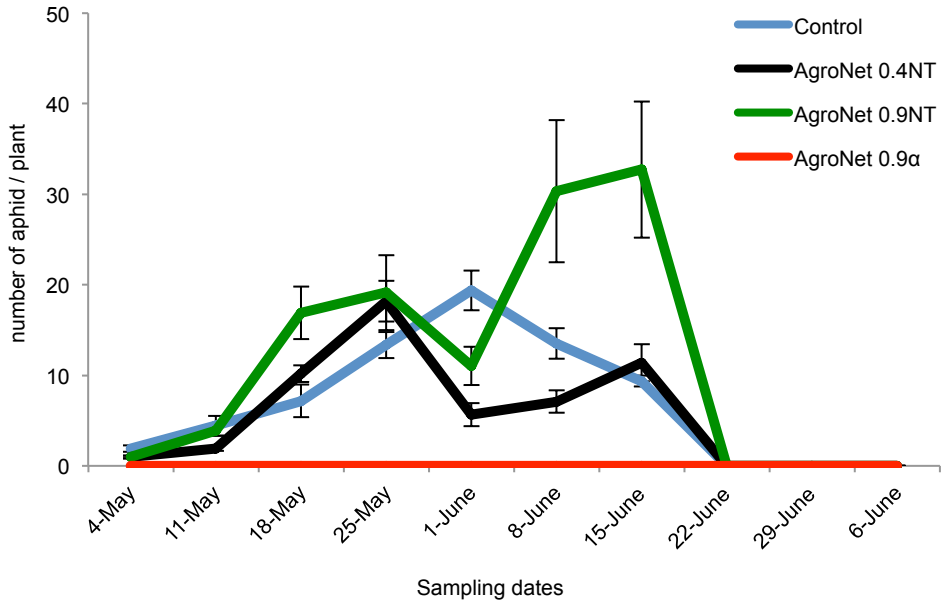

Figure 3. Evolution of aphid adults and larva on cabbage protected by nets. Number of aphids (±se) from *B. brassicae*, *M. persicae* and *L. erysimi* observed on 21 cabbages covered permanently with an alphacypermethrin treated net (AgroNet 0.9α) or a non-treated net (AgroNet 0.9NT or AgroNet 0.4NT) in comparison with a non-protected control.[77]

Cependant au Bénin comme au Kenya, si l'efficacité au champ des filets imprégnés d'alphacyperméthrine tant sur chou que sur tomate a été significativement supérieure au control en terme de protection contre les insectes piqueurs, elle n'a pas été suffisante pour justifier leur

[77] Martin et al 2013. Journal of Economic Entomology (submitted)

recommandation. Cela pourrait s'expliquer par le fort niveau de résistance aux pyréthrinoïdes des insectes piqueurs. Des essais sont en cours avec des filets imprégnés de permethrine réputé plus répulsif mais aussi avec des composés naturels ou non toxiques. En ce qui concerne l'effet des filets répulsifs sur les lépidoptères, les filets imprégnés d'alphacyperméthrine ont permis de réduire significativement le nombre d'œufs pondus par *P. xylostella* sur et à travers les filets. Des études menées à l'université de Catanes, Italie, ont confirmé l'effet répulsif de ces filets sur *Tuta absoluta* un dangereux ravageur de la tomate venu récemment d'Amérique latine. Ces filets pourraient être utilisés sur les ouvrants des serres/tunnels pour réduire leur taux de passage.

Les filets anti-insecte se sont révélés être une barrière efficace pour protéger les tomates contre la noctuelle *H. armigera* mais aussi contre les oiseaux. Si la barrière visuelle de ces filets (agronet 0.4) contre les pucerons, les aleurodes et les thrips a été confirmée au champ en particulier au Kenya, cette protection n'a pas été suffisante au Bénin pour réduire significativement l'incidence des phytovirus transmis aux plants de tomate par les aleurodes. Malgré leur effet répulsif, les filets imprégnés d'alphacypermethrine n'ont pas permis d'améliorer suffisamment l'efficacité de cette barrière physique contre ces insectes piqueurs pour réduire significativement les symptômes de maladie virales et augmenter la production. Des études sont en cours avec des filets imprégnés de permethrine et d'autres produits répulsifs non toxiques.

5.6.2 Impact micro-climatique

Dans la zone littorale du sud Bénin où nous conduisons nos expérimentations les conditions de fortes température et d'humidité ne sont pas toujours propices à l'utilisation de filet à petites mailles (0,4 à 0,9 mm). En saison sèche (mini-maxi : 22-40°C et 45-100%HR) les plus fortes températures sous filet peuvent provoquer une réduction du poids et de la taille des choux. En saison des pluies (mini-maxi : 22-30°C et 75-100%HR) les forts taux d'humidité peuvent provoquer des pourrissement des choux et des pullulations d'escargots. Des études sont en cours avec des filets gris avec des mailles plus larges. En attendant l'ouverture 1 à 2 fois par semaine des filets pendant la journée permet d'éviter ces problèmes. Même si cela est couteux en temps, les filets, réutilisables pendant plusieurs années, permettent de bien protéger les pépinières, de réduire le nombre de traitements insecticides, d'augmenter la production en qualité et quantité.

Au Kenya par contre, nous avons montré dans la région de Nakuru (1850 m) que les filets réduisent la température ambiante diurne et augmentent la température ambiante nocturne. Le taux moyen d'humidité du sol est significativement plus élevé sous filet ce qui permet de réduire l'irrigation. Ces conditions micro-climatiques ont pour conséquence un meilleur développement des plantes. Ajouté à la barrière anti-insecte, nous avons obtenus avec les filets une amélioration significative de la production en tomate, oignon, haricot vert et chou tant en qualité qu'en quantité. En ce qui concerne les pépinières sous filet tant pour le chou[78] que la tomate[79] les résultats sont remarquables. Ces résultats peuvent expliquer la tentation des maraîchers à utiliser

[78] Gogo et al 2012. *Hort Technology*, 22, 292-298
[79] Muleleke et al 2013. *Agronomy* 3, 2.

leur moustiquaire de lit pour protéger leurs pépinières.

En effet, dans les hautes terres du Kenya les pépinières de chou sous filet ont subi une température et une humidité relative plus élevées que sans filet (Figure 4). La croissance des semis a été plus rapide avec une réduction des dommages des insectes nuisibles. La germination des graines et la levée des semis a été plus précoce. Le taux de germination des graines a été plus élevé ainsi que le taux de survie des plantules, ce qui indique un potentiel de réduction du nombre de graine à semer. Les plants cultivés sous filet ont une conductance stomatique plus élevée et les feuilles contiennent plus de chlorophylle. Ils ont grandi plus vite, avec plus de feuilles et dans un court laps de temps par rapport aux plants témoins. L'utilisation d'un filet sur une pépinière de choux offre une technologie durable pour améliorer la performance des semis tout en réduisant les infestations de ravageurs, réduisant ainsi le coût de production, améliorant ainsi le revenu du producteur.

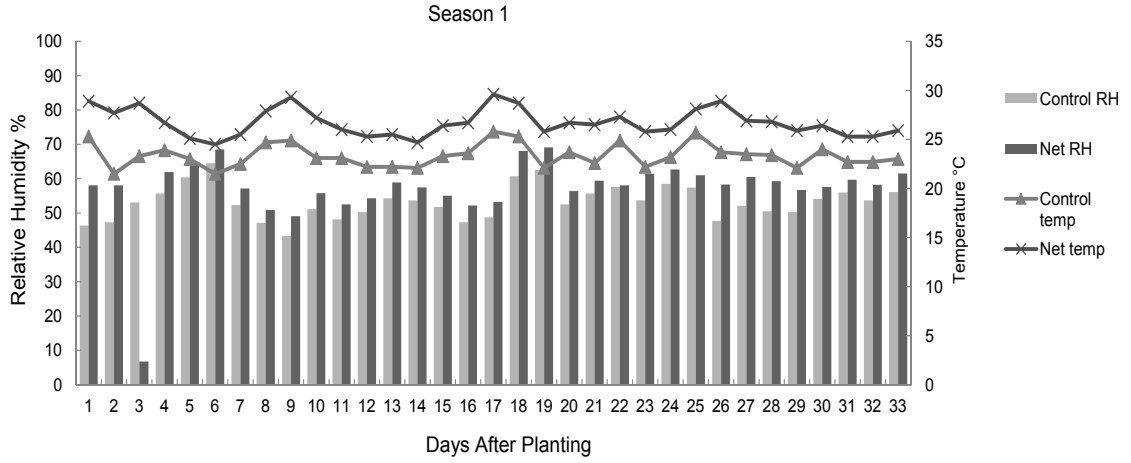

Figure 4 : Température et humidité relative d'une pépinière de chou avec et sans filet sur la station d'Egerton University, Njoro, Kenya de mars à avril 2011.

5.6.3 Conclusion sur les filets et perspectives

En Afrique subsaharienne, les insectes et les acariens herbivores constituent le principal danger pour les cultures maraîchères car il n'y a pas de saison froide pour réduire ces populations. Parmi ces ravageurs, les chenilles de lépidoptères sont les plus dangereuses car elles détruisent directement les feuilles, les fleurs, les fruits. Une chenille d'Helicoverpa par exemple peut détruire la production d'un plant entier de coton, de tomate ou de chou. Les filets permettent de contrôler efficacement la plupart de ces noctuelles. Pour celles qui pondent sur ou à travers les filets, l'imprégnation avec un produit répulsif ou irritant peut être une solution. Quoiqu'il en soit la protection des légumes contre les attaques de chenilles permet de supprimer le recours aux traitements chimiques en particulier ceux à base de pyréthrinoïdes. Les avantages des pyréthrinoïdes c'est qu'ils ont un large spectre, ils sont à la fois irritants et toxiques à faible dose, ils sont bons marchés et leur toxicité sur les mammifères est très faible. Le problème avec les pyréthrinoïdes c'est que, du fait de leur large spectre d'efficacité et de leur rémanence, ce sont des armes de destruction massive responsables d'importants dommages collatéraux en particulier sur les insectes utiles tels que les parasitoïdes et les prédateurs mais aussi sur la faune aquatique.

Par ailleurs leur utilisation systématique en agriculture depuis une trentaine d'années a sélectionné des populations résistantes surtout parmi les ravageurs. Cela a entrainé une augmentation des doses et des fréquences d'application et sélectionné des mécanismes de résistance multiples. Leur suppression des systèmes de culture maraîchers rendue possible par l'utilisation des filets pourrait permettre un retour progressif des populations d'insectes utiles et d'acariens prédateurs. Car ces insectes et acariens sont généralement capables de réguler les populations d'insectes piqueur-suceurs (aleurodes, pucerons et thrips) dont les dégâts directs ont peu d'impact sur la production dans la mesure où ils ne pullulent pas. Le principal danger de ces insectes piqueurs est leur capacité à transmettre des phytovirus qui eux ont un impact direct sur la production. Rien qu'un retard dans l'inoculation de ces virus peut améliorer la productivité des plantes d'où l'intérêt de bien protéger les pépinières puis les jeunes plantules. Après le repiquage, l'intégration de filets anti-insecte à la stratégie de protection des cultures permettrait donc d'additionner l'effet des processus de régulation naturels (insectes utiles, acariens prédateurs) à plusieurs techniques biologiques qui leurs sont compatibles : plantes répulsives, entomopathogènes, tolérance variétale… le tout dans le cadre d'une agriculture écologiquement intensive. C'est cette hypothèse de recherche que nous allons essayer de valider dans les trois projets de recherche suivants.

6 Projets de recherche en cours : L'écologie chimique au service des stratégies IPM

D'après Griffon (2013), le projet de l'écologie intensive est une tentative qui consiste à proposer des techniques qui s'insèrent dans le cadre des fonctionnements naturels : amplification du fonctionnement des fonctionnalités naturelles, complexification et usage de la diversité biologique comme source de résilience, imitation des processus naturels par bioinspiration (inspiration scientifique découlant de l'imitation de processus naturels) et biomimétisme (imitation de formes naturelles par des techniques). L'écologie chimique est la discipline de l'écologie qui étudie les rôles des médiateurs chimiques dans les interactions entre espèces vivantes, animales ou végétales. Les recherches que nous conduisons à l'icipe dans cette discipline devraient donc nous permettre d'intégrer nos stratégies de protection des cultures horticoles dans un projet d'écologie intensive:

- En recherchant des produits non toxiques répulsifs dans les plantes qui pourront à leur tour être sélectionnées pour cette propriété afin d'être utilisés en association ;
- En recherchant des attractifs de prédateurs émis pas les herbivores et des attractifs d'herbivores émis par les plantes afin de mieux pouvoir les attirer ou les piéger ;
- En étudiant le comportement des ravageurs et de leurs prédateurs.

Ces recherches sont conduites principalement au centre de recherche d'Icipe mais aussi en partenariat avec l'IRD-UMR Mivegec et le CREC pour les tests sur moustiques, le CNRS-UMR Cefe, l'USDA-Montpellier et le Cirad-UR Hortsys, l'INRAB, le KARI et l'université d'Egerton pour les tests sur aleurodes. Nous travaillons également en collaboration avec les sociétés L2I et A to Z textile Mills pour imprégner et produire nos filets expérimentaux.

6.1 Des filets répulsifs pour protéger les plantes contre les insectes vecteurs

Si l'intérêt des filets répulsifs pour protéger les cultures a été montrée avec des filets imprégnés d'alphacyperméthrine, la résistance de la plupart des ravageurs à ces pyréthrinoïdes de synthèse nécessite de trouver d'autres produits alternatifs non toxiques pour éviter tout risque de pollution. Depuis décembre 2011, j'encadre et co-dirige la thèse d'Emilie Delétré, dont l'objectif est de rechercher et de caractériser des produits répulsifs à partir d'une vingtaine d'extraits de plantes réputées répulsives ou de composés synthétiques non toxiques. Deux insectes modèles ont été retenus pour étudier leur comportement à l'égard de ces produits en raison de leur intérêt pour la santé humaine et la santé des plantes à savoir : le moustique *Anopheles gambiae*, vecteur du paludisme et l'aleurode *Bemisia tabaci*, vecteur de phytovirus (ex : TYLCV). L'intérêt de travailler en parallèle sur ces deux insectes modèles est leur complémentarité. En effet la majorité des études réalisées sur les répulsifs a été faite sur moustiques de même que les études sur les filets ou moustiquaire imprégnés de pyréthrinoïdes (toxique et excito-répulsif). Il en est de même pour le comportement du moustique à l'égard des composés volatiles. L'originalité de ces recherches a d'abord consisté à caractériser les propriétés répulsives, irritantes et toxiques d'une vingtaine d'extraits de plante et de chacun de leurs composés majoritaires à l'égard de ces deux insectes. Nous avons ainsi montré que certains produits pouvaient être répulsifs et/ou irritants et/ou toxiques sur *B. tabaci* et/ou *An. gambiae* et que ces effets étaient dose-dépendant. Nous avons montré également que les propriétés répulsives ou toxiques des extraits de plante pouvaient être dues à une seule molécule ou à un mélange de molécules. Ces bioessais ont été également conduits sur quatre molécules de synthèse non toxiques dont certaines ont montré des propriétés répulsives et/ou irritantes prometteuses. Un autre résultat indirect est d'avoir montré que les pyréthrinoïdes utilisés dans nos témoins positifs n'ont pas de propriété répulsive à distance mais après contact du fait de leur effet irritant (et excitant). C'est donc plutôt ce type de propriété qui sera recherché chez les produits candidats pour imprégner des filets anti-insectes.

Suite aux bio-essais conduits au laboratoire, deux à trois produits seront retenus pour être imprégnés sur des filets et des moustiquaires par les chimistes de la société L2I. Ces filets appliqués sur tomate seront testés sur *B. tabaci* sur la station expérimentale du KARI au Kenya. Les filets de types moustiquaire seront testés sur *An. gambiae* en tunnel avec cobaye au LIN-IRD de Montpellier. Nous chercherons également à valider l'hypothèse d'une combinaison synergique des filets et des volatiles répulsifs pour protéger des plants de tomate, contre l'aleurode *B. tabaci*. Ces filets constituants déjà une barrière visuelle, l'émission des composés volatiles à l'intérieur ou à l'extérieur des filets pourrait permettre de mieux repousser les insectes piqueurs ou de réduire l'attractivité des plants de tomate en masquant leur odeur déjà confinée sous le filet. Le suivi des populations d'insectes sur les plants de tomate d'une part, la collecte et l'analyse des composés volatiles à différentes périodes de la journée d'autre part, devraient nous permettre de vérifier cette hypothèse. Enfin les mécanismes de perception et d'action des produits candidats seront étudiés sur *An. gambiae* ou *B. tabaci*.

6.2 Une nouvelle stratégie push-pull pour lutter contre le thrips du niébé

Le thrips, *Megalurothrips sjostedti* est un ravageur majeur des cultures de haricot en Afrique (Ekesi *et al.,* 1998). Il peut causer de 20 à 100% de perte de production sur le niébé (*Vigna unguiculata* L.) en s'attaquant principalement aux fleurs (Ekesi and Maniania, 2000). La stratégie de protection utilisée par les agriculteurs au Kenya repose principalement sur la lutte chimique. Mais ces dernières années cette lutte chimique a montré ses limites du fait de l'augmentation des populations de thrips aux champs résultat sans doute de la sélection de populations résistantes. Il y a donc une nécessité de développer une stratégie IPM adaptée à la technicité des agriculteurs et moins dépendante des pesticides. Dans le cadre d'un projet financé par l'Union Africaine, j'encadre donc un doctorant ethiopien, Andnet Bayleygn Abtew, dont l'objectif sera d'élaborer une nouvelle stratégie push-pull pour lutter contre le thrips du niébé *M. sjostedti*. Cette stratégie sera basée sur l'utilisation de plantes répulsives qui seront associées au niébé et de pièges attractifs placés à l'extérieur et autour du champ. Ces pièges attractifs sont composés d'une phéromone et d'un champignon entomopathogène *Metarhizium anisopliae* souche 69 commercialisé au Kenya. En cas de forte infestation, des traitements foliaires à base de produits répulsifs seront associés à des bandes de polyéthylène bleues imprégnées d'insecticide spécifique placées le long des lignes de traitement.

Des recherches seront conduites dans le laboratoire d'écologie chimique de l'Icipe pour rechercher des composés volatiles répulsif du thrips *M. sojestdi* à partir d'huiles essentielles mais aussi de plantes candidates présentes dans la région. L'identification des composés organiques volatiles (COV) répulsifs agissant seuls ou en mélange et de plantes répulsives locales dont on aura caractérisé la composition en COVs permettra ensuite de valider cette stratégie push x pull sur le terrain avec les agriculteurs. Dans le même temps des bioessais seront conduits pour tester l'efficacité insecticide des bandes bleues imprégnées d'insecticide. Lors de son application au champ on évaluera l'impact des traitements répulsifs associés aux bandes imprégnées de toxiques. Au final la stratégie push x pull de fond (plante répulsive + piège attractif olfactif) sera renforcée ponctuellement par une autre stratégie push x pull de choc (traitement répulsif + piège attractif visuel).

6.3 Etude du comportement de l'acarien invasif *Tetranychus evansi* pour le piéger

T. evansi est un acarien originaire du Brésil. Il a été observé pour la première fois en Afrique de l'Est à la fin des années 90s et dans les années 2000 en Afrique de l'Ouest. En l'absence d'un prédateur efficace il est devenu rapidement un des ravageurs majeurs des Solanacées et en particulier de la tomate et de l'aubergine africaine (*Solanum* spp). Il est extrêmement prolifique et au Bénin en particulier il semble avoir pris la place des autres espèces d'acariens. Certaines populations de *Phytoseilus longipes* originaires du Brésil ont été identifiées comme étant des bons prédateurs de *T. evansi*. Pour cette raison il a été introduit au Kenya il y a quelques années. Mais aucune initiative dans ce sens n'a encore été prise en Afrique de l'Ouest où il peut occasionner des dégâts considérables.

L'étude du comportement migratoire et de dispersion de cet acarien sur plant de tomate devrait nous permettre de piéger cet acarien en particulier sous les filets anti-insecte où il peut facilement proliférer. C'est l'un des objectifs de la thèse de Ginette Azandemé que j'encadre et

co-dirige avec le Pr Serge Kreiter. Le second objectif de cette thèse est d'identifier un ou plusieurs composés attractifs du prédateur *P. longipes* et d'autres de l'acarien *T. evansi*. En effet, la disposition d'un composé attractif de *P. longipes* qui serait émis par sa proie *T. evansi* nous permettrait de sélectionner puis de multiplier une souche de prédateurs spécifiques. Cela pourrait nous permettre également d'attirer ce prédateur dans les cultures. En revanche la disposition d'un composé attractif de *T. evansi* nous permettrait de renforcer l'efficacité d'un piège à acarien. En effet nous avons déjà élaboré et breveté un filet acaricide très efficace pour piéger les acariens *Polyphagotarsonemus latus* et *T. urticae* (Martin et al 2010). Cependant le filet acaricide n'est pas adapté aux cultures tuteurées comme la tomate. Ce piège qui reste encore à élaborer pourrait être constitué d'un morceau de mousse imprégné du champignon entomopathogène *Metarhizium anisopliae* qui serait fixé sur les tuteurs de tomate. En effet l'action de ce champignon a déjà été montrée sur *T. evansi* et G. Azandeme a montré récemment la migration circadienne ascendante et descendante de *T. evansi* comme cela avait été montré auparavant pour *T. urticae* (Pralavorio et al 1986). L'hypothèse de recherche que nous voulons confirmer est qu'une proportion significative d'une colonie de *T. evansi* présente sur un plant de tomate effectue des migrations ascendantes et descendantes. Ainsi ces acariens pourraient se charger en spores de champignons et pourraient ainsi infecter régulièrement le reste de la colonie et cela d'autant plus que nous avons montré le comportement très grégaire de cet acarien contrairement à *T. urticae* qui se disperse rapidement sur toute la plante.

6.4 Projet Filet et impact Microclimatique : BioNetAgro II

L'objectif principal du projet BioNetAgro est de développer l'utilisation des filets anti-insectes sur les cultures de chou et de tomate pour les petits producteurs maraîcher du Bénin et du Kenya afin de réduire le recours aux insecticides et d'améliorer la qualité de la production. Ce projet financé en octobre 2010 par le département Hort CRSP USAID (500 k$) prendra fin en octobre 2013. L'université de l'état du Michigan, MSU (M. Ngouajio) et le Cirad (T. Martin) en assurent le leadership. Ce projet est réalisé par une équipe internationale (USA, France, Kenya, Benin, Tanzanie), pluridisciplinaire (agronomie, entomologie, physiologie de la plante, sociologie, économie) et multi-institutionnelle (MSU, Cirad, KARI, Icipe, Univ. Egerton, INRAB, Univ. Abomey Calavi, ONG). La société privée tanzanienne, A to Z Textile Mills produit et fournit les filets anti-insecte. Les recherches sont conduites à trois niveaux (laboratoire, stations expérimentales et champs). Fort de ce dispositif les résultats obtenus ont confirmé l'efficacité de la barrière physique contre les ravageurs majeurs entraînant une réduction de 70 à 100 % des traitements insecticide sur chou et une modification des conditions micro-climatiques pouvant entrainer une augmentation de la productivité des pépinières et des tomates, mais aussi une réduction des besoins en arrosage (2 articles publiés, 6 articles en préparation). Une vingtaine d'étudiants ont réalisés leur stage de licence et de master sur ce projet. Et plus de 360 maraîchers ont été formé directement ou indirectement à l'utilisation de cette technique tant au Bénin qu'au Kenya. Le processus d'adoption de cette technologie par les petits maraichers fait l'objet de deux thèses de doctorat en socio-économie conduites l'une au Bénin et l'autre au Kenya. Ces travaux permettront également d'évaluer l'intérêt économique et environnemental de cette nouvelle technique pour les petits maraîchers. Car en effet, si cette technique assure une protection efficace des plantes, qu'elle apparaît facile à utiliser, qu'elle est sans risque pour la santé

humaine et pour l'environnement car réutilisable et recyclable, qu'elle peut être combinée avec d'autres méthodes de lutte dans le cadre d'une agriculture biologique ou raisonnée, de nouvelles contraintes apparaissent liés à l'arrosage, la manipulation des filets, leur stockage, leur recyclage et certains problèmes ne sont pas résolus comme le contrôle des insectes vecteurs de phytovirus. Par ailleurs l'utilisation des filets n'est pas facilement transposable aux plantes pollinisées par les insectes.

C'est pourquoi une seconde phase du projet est en cours d'élaboration pour améliorer l'efficacité des filets anti-insecte pour la protection contre les insectes vecteurs (pucerons, aleurodes, thrips), les adapter à la protection d'autres cultures horticoles (haricot vert, fraises, courgettes, melon,…), les combiner à d'autres méthodes de lutte biologique (association de culture, plantes répulsives, entomopathogènes, insectes utiles) et à d'autres techniques culturales telles que la micro-irrigation et la couverture du sol. En fonction des conditions climatiques et du spectre parasitaire nos recherches porteront également sur les structures et la couleur des filets. Les thèses en cours portant sur la recherche de composés ou de plantes répulsives devraient permettre d'améliorer l'efficacité des filets pour protéger les plantes contre les insectes vecteurs de phytovirus et les acariens phytophages. Le partenariat public/privé que nous avons construit avec la société tanzanienne A to Z Textile Mills dans le cadre d'un MOU signé avec le Cirad nous donne une grande marge de manoeuvre pour développer des filets pouvant être adapté à chaque culture selon leur cortège parasitaire et les conditions climatiques locales. Cette société qui produit depuis 2003 des moustiquaires imprégnées pour tout l'Afrique de l'Est a l'avantage d'être présente en Afrique et de vouloir développer ses activités dans le secteur agricole à travers la production de filet anti-insectes, de sac de stockage de grains et du matériel de micro-irrigation.

6.5 Projet de réseau Maraîchage et Santé en Afrique subsaharienne : MARSA

L'agriculture autour des grandes villes africaines est dominée par le maraîchage. Cette filière approvisionne les populations urbaines en légumes qui jouent un rôle important dans l'alimentation et la prévention de l'obésité, du diabète, des maladies cardio-vasculaires... Le maraîchage fournit par ailleurs un revenu à de nombreux petits producteurs et leurs manœuvres, mais aussi en amont (fournisseurs d'intrants agricoles) et en aval (des grossistes aux détaillants). Mais le maraîchage périurbain est également consommateur d'intrants que ce soit les pesticides pour lutter contre les nombreux bioagresseurs ou les engrais et amendements pour améliorer la fertilité des sols. Mal employés ces intrants mettent en danger la santé des agriculteurs qui les manipulent, celle des consommateurs qui peuvent ingérer des teneurs résiduelles élevées de pesticides et de nitrates voire pour l'ensemble de la population urbaine à travers la pollution des nappes phréatiques et la prolifération de vecteurs de maladies infectieuses : paludisme, dengue et chikungunya.

La prise de conscience des risques sur la santé et sur l'environnement est à l'origine de la conception avec les petits maraîchers de nouveaux systèmes de culture adaptés, durables et plus respectueux de l'homme et de la nature. Ces deux aspects sont d'autant plus importants que la population urbaine d'Afrique Sub-saharienne augmente très rapidement entrainant une demande

croissante en légumes frais. Or l'absence de contrôle qualité tant des intrants utilisés que des légumes mis sur le marché, nécessite un travail de recherche en amont pour élaborer, avec les maraîchers, des systèmes de culture durables. Ces problématiques communes à toute l'Afrique Sub-saharienne et l'isolement des acteurs impliqués dans la recherche de solutions adaptées, nécessite la mise en place d'un réseau international dynamique pour mettre en commun les idées, les compétences, les savoirs faire, regrouper les besoins en formation et élaborer des projets de recherche et de développement.

Pour partager ces résultats et ces innovations avec les différents acteurs qui travaillent dans la filière maraîchage en Afrique Subsaharienne et les adapter aux différentes conditions climatiques et de parasitisme nous avons élaboré un projet de réseau : le réseau MARSA (Maraichage et santé en Afrique Subsaharienne). Suite à la dévaluation du franc CFA et aux politiques d'ajustement structurel imposée par le FMI et la Banque Mondiale aux pays d'Afrique Subsaharienne au début des années 90s, les centres de recherche agronomique n'ont cessé de décliner faute de fonds de soutien et d'un renouvellement des ressources humaines. Depuis quelques années cependant de jeunes équipes de recherche ont montré leur dynamisme à travers le montage et la réalisation de projets internationaux. De leur coté, les centres de recherche internationaux comme le Cirad, l'IRD et autres CGIAR, travaillant en partenariat avec ces centre nationaux ont fortement contribué à former un vivier de jeunes chercheurs. L'objectif de ce réseau MARSA est de mettre en commun les savoirs faires et les connaissances mais aussi de permettre des complémentarités entre ces jeunes équipes africaines qui ne disposent pas toujours des compétences ou du matériel requis dans toutes les disciplines pour proposer ensemble des projets de recherche et de développement.

Le réseau MARSA, pour une agriculture maraîchère durable respectueuse de l'environnement en Afrique Sub-saharienne, associe des compétences scientifiques en sociologie, en entomologie, en agronomie, en santé publique et en économie. Il se base sur un partenariat international multiple et varié qui a fait ses preuves à travers la création de réseaux locaux et la réalisation de projets de recherche communs. Il regroupe les institutions suivantes : UAC, INRAB, IRSP, CREC au Bénin ; Université de Ouagadougou, IRSS au Burkina Faso ; CNRA en Côte d'Ivoire ; UCAD au Sénégal ; Université de Lomé au Togo ; Icipe, Egerton University au Kenya ; Cirad Hortsys, Ird UMR Mivegec en France.

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8 Publications les plus importantes

HORTICULTURAL ENTOMOLOGY

Efficacy of Mosquito Netting for Sustainable Small Holders' Cabbage Production in Africa

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ABSTRACT The efficacy of a mosquito netting to protect cabbages, *Brassica oleracea* L., against pests was investigated in field trials in Benin, West Africa. A polyester net covered the plants at night by using a wood armature. The net was removed during the day to prevent overheating and excessive shade, both problems of insect-proof screens used under tropical conditions. The number of all lepidopteran larvae with netting protection and foliar insecticide sprays was significantly lower than the unprotected control. The number of diamondback moth, *Plutella xylostella* (L.), was significantly lower with netting protection compared with foliar insecticide sprays and control. Netting treated with deltamethrin gave total protection of young plants against the aphid *Lipaphis erysimi* (Kaltenbach). At harvest, the number of marketable cabbages protected with untreated netting was significantly higher compared with the production with foliar insecticide sprays. The protection of cabbages with netting can be an economically viable method. Considering the price of cabbages on local markets (US$1/unit), the net returns per 100 m^2 were US$247 by using netting, US$149 by using insecticides, and US$117 for controls. The net returns for using netting are based on replacing the netting each crop cycle. But netting can be reused several times, depending upon conditions, increasing the profit margin. The netting protection may be an alternative to the growing unsustainable practices of vegetable cropping in peri-urban areas of tropical countries.

KEY WORDS vegetable protection, mosquito netting, insecticide treated net, *Plutella xylostella*, cabbage

Farmers in Sub-Saharan Africa use a large amount of pesticides on vegetables, and the use is exacerbated by insecticide resistance. Small-scale producers rarely have access to training in pesticide use and have only limited, or no access, to advice on the management of pesticides (Dinham 2003). Without a thorough knowledge of alternatives, farmers often assume that the only solution to pest problems is to increase dose and spray frequency. Moreover, the reentry periods after spraying and withholding periods are not known. The insecticides used are often very hazardous to human health, affecting users, produce consumers, and the environment. Pesticide residues from agricultural use select for resistance in mosquitoes, threatening the efficiency of those insecticides that also are used for mosquito control such as malaria vectors (Diabate et al. 2002). These unsustainable pesticide use practices are increasing with the demand for vegetables in expanding African cities.

To reduce insecticide pollution, a solution is to replace foliar insecticide sprays with an insect-proof net covering vegetables. This technique has been used with success in China to protect cabbage and in the Netherlands on various vegetables (Ester et al. 1994, Chen et al. 1998). In West Africa, insect-proof nets and particularly insecticide-treated nets, have only been used as bed-nets in public health to prevent malaria morbidity and mortality (Hougard et al. 2002). Therefore, these nets, treated or untreated, and netting fabrics are readily available in local markets.

The aim of this study was to adapt netting techniques to small-scale farming practices, testing insect-proof netting (insecticide treated or untreated), for the protection of cabbage, *Brassica oleracea* L., crops in peri-urban areas of Cotonou, Benin. Insecticide-treated netting was used to protect seedling plants, and untreated netting was used to protect cabbage crops after planting-out. Insecticide-treated net was limited to young plants to protect from harmful attacks of small pests. Results were compared with the local foliar insecticide protection recommended by the National Research Institute of Agriculture of Benin (INRAB). Cabbage was chosen because it is one of the most important cash crops among vegetables in Africa, and it attracts a wide range of pests, leading to important yield losses (Goudegnon et al. 1998). Cabbages are therefore heavily treated by insecticides and often with inappropriate and hazardous chemicals. We planned to remove the net during hot periods of the day when the flight activities of pests were reduced to suppress the problems of overheating and

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Table 1. Number of all caterpillar species, percentage of aphid-infested plants and percentage of damaged plants in seedling cabbage nurseries with insecticide-treated netting and untreated netting compared with foliar insecticide sprays and unprotected controls

Treatment	Caterpillars/10 plants[a]	% aphid-infested plants[b]	% damaged plants[c]
Control	6.3 ± 1.9d	20.0 ± 18.7ab	16.6 ± 7.1c
Foliar insecticide sprays	4.2 ± 1.2c	22.5 ± 25.2ab	21.1 ± 10.3c
Untreated netting	1.7 ± 1.0b	52.5 ± 36.7b	6.6 ± 5.1b
Insecticide-treated netting	0.0 ± 0.0a	0.8 ± 2.0a	2.0 ± 2.8a

Means ± SEM in the same column with the same letter are not significantly different ($P < 0.01$).

[a] Cumulative number for the total of all caterpillar species from five samples, including *H. undalis, S. littoralis, P. xylostella, T. ni,* and *Agrotis* spp.

[b] Average of three samples.

[c] Percentage of damaged plants just before planting-out (100 plants observed per plot).

excessive shade with insect-proof screens used in tropical conditions (Desmarais 1996).

Materials and Methods

Seedling Cabbage. The trial with a local variety of cabbage was implemented in the Research Centre of Agonkanmey of INRAB. Four treatments with three replicates were compared: insecticide-treated net, untreated net, foliar insecticide sprays, and unprotected control. The plots (1 by 2 m) were randomized in a complete randomized block design. Four rows of cabbages were sowed in each plot in November at the beginning of the dry season. Insecticide-treated nets and untreated nets were simple white bed-nets from local market in knitted polyester, 30 g/m^2 and 25 holes per cm^2. Six bed-nets (1 by 2 by 1.5 m) were cut down to be 75 cm in height. Three treated nets were insecticide impregnated 2 d before use, by dipping in deltamethrin formulation at a rate of 50 mg (AI)/m^2. Four wood pickets at each corner kept the nets at a height of 50 cm over seedling plants.

Planting-Out. Cabbages were planted-out in the field 20 d after sowing in nine plots of 6 m^2 (1.2 by 5 m) randomized in complete randomized block design with three replicates. Plots were three rows of 12 plants that have been previously protected by an insecticide-treated net. Three treatments were compared: untreated netting, foliar insecticide sprays, and unprotected control. Three untreated nets (2 by 6 m) were made from white mosquito netting fabric. The nets were put on a light wooden frame above the cabbage plots to protect young plants. Nets were used only at night to avoid plant overheating problems and to facilitate manual watering. Nets were put in place every day at 5 p.m. and removed the next morning at 9 a.m.

Pesticides. Cabbage seeds were treated with a formulation of Super Homai 70 DS (thiophanate-methyl thiram diazinon used at 50 g [AI]/kg seeds) from Nippon Soda (Tokyo, Japan) for a protection against soil insects, nematodes, and fungus. Deltamethrin 25 EC from ALM (Abidjan, Côte d'Ivoire) was used for foliar sprays and net impregnation. In keeping with local recommendations, deltamethrin was used at 12 g (AI)/ha for foliar sprays. Two foliar insecticide sprays were applied on seedling cabbage. Then, 10 foliar insecticide sprays were applied on cabbages twice a week after planting-out. Foliar sprays ceased 2 wk before harvesting.

Sampling. In seedling cabbage, the number of caterpillars was counted on two samples of 10 plants per plot, twice a week. Individual species counted were *Hellula undalis* (F.), *Spodoptera littoralis* (Boisduval), *Trichopulsia ni* (Hübner), *Plutella xylostella* (L.), and *Agrotis* spp. The number of infested plants by the aphid *Lipaphis erysimi* (Kaltenbach) was recorded from two samples of 10 plants, twice a week. Before planting-out, the percentage of damaged plants was noted on 200 plants per replication. After planting-out, the same sampling was done once a week. The number of *P. xylostella* adults was counted on two samples of 20 plants per plot. The number of vegetables of good quality (with no or few damage) also was recorded during the sixth, seventh, and eighth week after planting-out on 20 plants per plot. Harvesting was done, row by row, separating out marketable cabbages, which were used for the yield calculations. The other cabbages were considered as losses because of poor quality.

Analysis. MINITAB software (Minitab, Inc., State College, PA) was used for statistical analysis. Analysis of variance (ANOVA) was done on cumulated data for caterpillars and on average data for aphids. Fisher and Mann–Whitney *U* tests were used for comparisons of means with 0.05 error rate.

Results

The major pests of seedling cabbage were the larval stages of Lepidoptera. The first caterpillar attack was observed around 15 d after sowing, principally on plots without netting. In unprotected control plots, the caterpillars were largely *H. undalis* (33%) and *S. littoralis* (33%); less abundant caterpillars were *T. ni* (15%), *P. xylostella* (10%), and *Agrotis* spp. (5%). Protection with netting, either treated or untreated, against caterpillar infestations was significantly more effective than foliar insecticide sprays and the unprotected control (Table 1). There were significant differences in cumulative sampled caterpillars per 10 plants between the four treatments with the greatest number on untreated controls, intermediate numbers on netted and sprayed cabbage, and no caterpillars detected on

Table 2. Number of larvae for each lepidopteran species, number of *P. xyllostella* adults, and percentage of aphid-infested plants in cabbage plots with untreated netting compared with foliar insecticide sprays and unprotected controls

Treatment	*H. undalis* larvae/ 10 plants[a]	*S. littoralis* larvae/ 10 plants[a]	*P. xylostella* larvae/ 10 plants[a]	*T. ni* larvae/ 10 plants[a]	*Agrotis* larvae/ 10 plants[a]	*P. xylostella* adults/ 10 plants[b]	% aphid-infested plants[c]
Control	14.2 ± 2.4b	3.3 ± 0.6b	0.8 ± 0.4	8.0 ± 4.9	0.7 ± 0.3	20.3 ± 3.1b	4.4 ± 1.8
Foliar insecticide sprays	4.2 ± 1.2a	1.0 ± 0.2a	1.5 ± 0.8	0.8 ± 0.5	0.2 ± 0.2	14.0 ± 3.2b	1.9 ± 0.4
Untreated netting	3.2 ± 1.6a	1.0 ± 0.0a	0.2 ± 0.2	0.5 ± 0.5	0.3 ± 0.2	4.5 ± 1.4a	5.8 ± 3.2

Means ± SEM in the same column with the same letter are not significantly different ($P < 0.001$).
[a] Cumulative numbers of each caterpillar species from nine samples.
[b] Cumulative numbers of *P. xylostella* from seven samples.
[c] Average of six samples.

plants covered with insecticide-treated net. No caterpillars or aphids were found on seedling cabbage protected with insecticide-treated netting (Table 1). Although untreated netting was effective against caterpillars, it did not protect against aphids. Thus, the percentage of infested plants by aphids was significantly higher with untreated nets than with insecticide-treated nets. The beneficial effects of treated and untreated nets on seedling cabbage quality before planting-out compared with foliar insecticide sprays and unprotected control were significant (Table 1). The percentage of damaged plants was significantly lower with insecticide-treated netting than with untreated netting. There was no significant difference between the foliar insecticide sprays and unprotected control.

After 20 d in seedling nurseries, the young cabbages were planted-out in plots. The infestation of caterpillars began 2 wk later and increased until harvest. The caterpillar species observed on cabbage leaves were *H. undalis* (54%) and *P. xylostella* (20%) with some *S. littoralis* (13%), *T. ni* (11%), and *Agrotis* spp. (2%). The number of caterpillars per 10 plants was significantly lower with netting and foliar insecticide sprays compared with the unprotected control (Table 2), confirming results obtained with netting in seedlings nurseries. The number of *P. xylostella* adult per 10 plants was significantly lower with netting protection compared with foliar insecticide sprays (Table 2). Except at the beginning of the infestation, the number of *P. xylostella* adult was always lower on cabbages protected by netting than on cabbages with foliar insecticide sprays or in the unprotected control. The percentage of good-quality cabbage (not damaged or slightly damaged) grown under netting protection was significantly higher at 6 (68%), 7 (70%), and 8 wk (53%) after planting-out than with foliar insecticide sprays (28, 7, and 12%, respectively). The untreated control plots produced no good-quality cabbage at the last sampling date. Statistical analysis of cabbage production showed significantly two-fold higher production of marketable cabbages per 100 m^2 with netting protection compared with foliar insecticide sprays (Table 3). The local insecticide protection with 10 sprays of deltamethrin did not produce more marketable cabbages than untreated control. The yield, in terms of weight of marketable cabbages, was significantly greater for the netting protection than with untreated control and intermediate with the foliar insecticide sprays yield (Table 3).

Discussion

Our study demonstrated that an insect-proof net can effectively protect cabbage plants from aphids and caterpillars. This method of control reduced insect numbers more and produced greater cabbage yield and quality than standard foliar insecticide sprays. Protection from insects was achieved with the netting in place only at night, when the temperature is lower and the flight activity of adult moths is higher. This approach minimizes the negative effects of netting on

Table 3. Cabbage production with untreated netting compared with foliar insecticide sprays and unprotected controls

Treatment	Marketable cabbage[a] (no./100 m^2)	% marketable cabbage	Yield (kg/100 m^2)
Control	117 ± 39b	21.9 ± 9.2b	80 ± 32b
Foliar insecticide sprays	156 ± 69b	28.8 ± 12.8b	103 ± 46ab
Untreated net	383 ± 42a	67.9 ± 7.8a	198 ± 22a

Means ± SEM in the same column with the same letter are not significantly different ($P < 0.05$).
[a] Marketable cabbage is not damaged or slightly damaged.

the crop due to shading and overheating in hot climates similar to those of Benin where our experiments were conducted. In seedling nurseries, netting protected young plants from caterpillar infestations, evidently by preventing the females from laying eggs on the cabbage leaves at night. The physical barrier of the netting alone seems effective against *H. undalis*, *S. littoralis*, *P. xyllostella*, *T. ni*, and *Agrotis* spp. but not against small pests such as aphids, which can go through the commonly available mesh size we tested (25 per cm^2). Treating the netting with an insecticide (deltamethrin) provided 100% protection against aphids in our study. The treated nets also were more effective at reducing damage by all insects to cabbage plants. Plants protected with insecticide-impregnated netting were ≈3 times less damaged compared with those with untreated netting, and the rate of production of good-quality plants was significantly better from plots with treated netting. In contrast to the effectiveness of treated netting, the production of young plants from seedlings protected with foliar insecticide sprays was no different from the unprotected control, showing the ineffectiveness of two sprays of deltamethrin.

The use of netting at night also provided protection from caterpillar infestations in the cabbage crop after planting-out. There were 10-fold fewer caterpillars with the netting than on unprotected controls. The netting provided suppression of *H. undalis* equivalent to 10 sprays of deltamethrin. For *P. xylostella*, the netting reduced adult numbers three-fold more effectively than the insecticide treatments. The poor control of foliar insecticide sprays for *P. xylostella* could be due to the suspected pyrethroid resistance in this pest in Benin (Goudegnon et al. 1998). Genetic resistance in *P. xylostella* to chemical insecticides and biopesticides is a worldwide problem (Ferre and Van Rie 2002, Sayyed et al. 2004). The low-level caterpillar infestations that did occur on cabbages protected by netting in our study could be due to adult moths immigrating from nearby unprotected plots during the periods when the netting was removed in the daytime. This possibility seems likely for *P. xylostella*, which flies principally throughout the night (Goodwin and Danthanarayana 1984) but can be observed flying erratically when disturbed in the daytime. Despite this risk of occasional oviposition when the nets are removed, the practice has three advantages that offset this risk: 1) avoidance of overheating and shading the crop, which could decrease photosynthetic efficiency; 2) facilitation of watering and cultural practices; and 3) increasing longevity of the netting by limiting its exposure to UV.

Insect control using netting is cost-effective. The number of marketable cabbages produced under netting was twofold higher than the number produced using insecticide sprays. The current price of cabbages in local markets is approximately US$1/unit. The net returns per 100 m^2 were US$247 by using netting (US$383–US$76 cost of netting), US$149 by using insecticide (US$156–US$7 cost of insecticide), and US$117 for controls. These returns estimates for using netting are based on replacing the net at each crop cycle. But netting can be reused several times depending upon conditions, increasing the profit margin. Netting manufacturers have developed long-lasting nets for mosquito netting, which were still effective after 3.5–4 yr (Guillet 2001). They are wash-resistant and release insecticide over time, maintaining activity for at least 4 yr. They are commercially available in drugstore (≈US$7–15/unit) and could be used for the protection of seedling nurseries against aphids. The labor cost of removing the netting for a 100-m^2 plot is negligible (US$0.12/d).

The protection of growing vegetables with a net, insecticide treated or untreated, could prevent unsustainable insecticide practices in peri-urban areas of tropical countries. Advantages are protection of human health by reducing hazardous insecticide sprays, reducing environmental pollution from insecticide residues (important material for mosquito insecticide resistance issues), and increasing effectiveness of crop protection by improving yield and crop quality. This crop protection technique also might be useful in controlling insecticide-resistant pests highly selected in small-scale farming. The netting technique is well adapted to farmers cultivating intensively small plots in peri-urban areas of big African cities. The material is available in local markets and is cost-effective because it can be easily used many times. Africans are already familiar with protection from insects via bednets through the national malaria control programs, which should help adoption of netting for pest control in vegetables.

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Biochemical determination of acetylcholinesterase genotypes conferring resistance to the organophosphate insecticide chlorpyriphos in field populations of *Bemisia tabaci* from Benin, West Africa

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ABSTRACT

Resistance to chlorpyriphos insecticide in *Bemisia tabaci* from a field population collected in Benin, West Africa was suggested with bioassay showing the presence of two sub-populations. Patterns of acetylcholinesterase (AChE) inhibition by the organophosphate chlorpyriphos-oxon were analyzed to estimate the number of possible genotypes with different sensitivity expected in three *B. tabaci* field populations collected in Benin. The analysis of inhibition patterns in these populations compared with four laboratory strains of *B. tabaci* using chlorpyriphos-oxon allowed the differentiation of three possible genotypes. In the reference strain SUD-S we detected two different acetylcholinesterases with different sensitivity to chlorpyriphos oxon suggesting the presence of two genes ace 1 and ace 2. The proportion of the insensitive enzyme (ace 2) was estimated to be 31%. In field populations we can detect two alleles at the same gene locus ace 1: one susceptible ace1S and one resistant ace1R. Both strains called Arizona University and Mexico-S2 have lost sensitive ace1S but the field populations from Benin clearly contained at least three genotypes confirming heterogeneous populations not completely resistant.

1. Introduction

The cotton whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), is a major pest of cotton and vegetable crops in Benin, West Africa. This pest causes damage both directly by feeding and indirectly through the excretion of honeydew and as a vector of virus diseases [1,2]. Many factors contribute to its population growth such as climatic conditions, host plant preference [3], elimination of beneficial species [4] and selection of insecticide-resistant populations [5–7]. Resistance results from presence of genotypes in a population that confers some degree of protection against toxins [8]. The selective advantage is so if selection pressure is maintained. Consequently, the proportion of the population having the resistant genotype will increase with successive generations. The resistance to organophosphates (OPs) in African populations of *B. tabaci* has already been detected in Burkina Faso [9], and positive cross resistance with other molecules has been already observed in this chemical group [10]. The demonstration of OP resistance and the identification of the mechanism involved in *B. tabaci* can help to control this pest in Benin.

Acetylcholinesterase (AChE) is a key enzyme in nerve transmission and belongs to the serine hydrolases. In many insects there are two genes encoding AChE. One of these genes is synaptic and involved in nerve impulse transmission. Its inhibition by insecticide is lethal, and consequently resistance may occur by modification of its active site architecture. The other gene is non synaptic and belongs to the class of scavenger proteins. Its inhibition is not lethal and consequently it is not involved in resistance. When located in the insect nervous system, AChE terminates nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine (Ach) and regenerates the excitability of synapses [11]. According to its key physiological role in nerve transmission, AChE is the target of various insecticides or natural toxins [12]. Different AChE substrates and inhibitors interact either with the catalytic active site or with a peripheral site located in the vicinity of the active site. Reactivation time is very different between ACh and insecticide: it is practically immediate when the enzyme is acetylated but it is very long (from various hours to several days) when it is carbamylated or phosphorylated [13]. Consequently, OPs and carbamate insecticides are considered as irreversible inhibitors of AChE not following typical Michaelis–Menten kinetics. They bind to the

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reactive serine (part of the catalytic triad) in AChE and phosphorylate the enzyme to a greater or lesser extent irreversibly, i.e. dephosphorylation is extremely slow [14]. The phosphorylated enzyme can no longer hydrolyze acetylcholine and intoxicated insects die from over-excitation due to the lack of ACh hydrolysis.

There are many reports of modifications to this enzyme which result in target-site resistance in insect species [15]. Examples include *Musca domestica* [16], *Anopheles stephensi* [17], *Ceratitis capitata* [18], *Drosophila melanogaster* [19] and *Aphis gossypii* [20]. Insensitive AChE has also been described as one of the major mechanisms of resistance to OP and carbamate insecticides in whiteflies [6,20–26]. A decrease in sensitivity to inhibition by carbofuran and monocrotophos was found in whitefly populations from Sudan, which had been heavily selected with dimethoate and monocrotophos. In populations from Guatemala, Nicaragua, and Turkey, this AChE insensitivity was also detected after exposure to monocrotophos [6]. However, the heterogeneity of AChEs in these populations was not established until Byrne and Devonshire [23] characterized them as two AChE variants differing in their sensitivities to paraoxon and azamethiphos, and later Moores et al. [27] also identified a third AChE in a *B. tabaci* strain from Arizona.

The aim of this study was to determine whether the resistance to chlorpyriphos insecticide observed with bioassay in a field population from Benin is due to AChE insensitivity and to determine the proportion of sensitive or resistant AChE in field populations compared with laboratory strains.

2. Materials and methods

All of the abbreviations used were: for the genes (ace 1 and 2), the proteins (AChE1S AChE1R and AChE2) and the neurotransmitter ACh.

2.1. Insects

Adults from seven populations of *B. tabaci* were used. Three field populations were collected in southern Benin: Ben-Ago (B2) collected from eggplant in Agonkanmey near Abomey Calavi, Ben-Pah (B3) collected from mint in Pahou and Ben-Boh (B4) collected from cotton in Bohicon. These specimens were stored immediately at −80 °C after collection. Samples of frozen adult whiteflies from four laboratory strains called SUD-S (B1), Arizona University (B5), Mexico-S2 (B6) and United Kingdom (B7), originated from Sudan, USA, Mexico and UK respectively, were supplied by Bayer CropScience Laboratory, Monheim, Germany. SUD-S (B1) is usually used as an insecticide susceptible reference strain but we could not use it for bioassay taking into account the risk of introducing a new population to Benin. For bioassays, adult whiteflies were regularly collected from cotton leaves in an untreated field located in the research station of Bohicon (Ben-Boh or B4).

2.2. Chemicals

The insecticide chlorpyriphos ethyl (Pyrical 480 EC) used for bioassay was supplied by Arysta lifescience (Pau, France). Chlorpyriphos methyl-oxon supplied by Cluzeau Info Labo (Bordeaux, France) with purity greater than 95% was firstly dissolved in alcohol, then in water before use for biochemical studies.

2.3. Bioassays

The leaf-dip bioassay method used was based on that by previous workers [10,28]. Cotton leaf disks (55 mm in diameter) were dipped (10 s) in aqueous solutions of insecticide. The leaf disk preparations were air dried for 20 min before use. Treated disks were laid, adaxial side down, on a bed of agar gel (7 g L^{-1}) in the base of a small plastic Petri dish of appropriate size. Control disks were dipped in distilled water only. Twenty adult *B. tabaci* (mixed sex) were removed from the leaves of a cotton plant in the field with a mouth aspirator and placed into a small plastic vial. Then the vial was put into a freezer at −20 °C for 1 min 20 s before being placed onto the treated leaf disk. Each unit was then sealed with a transparent, close fitting, ventilated lid. When adult recovered, from narcosis, the dishes were stored upside down so the adults could assume their natural feeding position. All tests were carried out at 24.5 ± 1.5 °C and 43.2 ± 7.7% RH and final mortality was scored after 48 h using a binocular microscope. Individuals which showed signs of movement were considered alive. Each bioassay was replicated six times.

2.4. Measurement of AChE activity

Three enzyme preparations were done for each population by homogenizing 150 adult whiteflies (unsexed) in 300 μL 25 mM phosphate buffer pH 7.2, containing 1 mg/mL bovine serum albumin. Samples were centrifugated for 10 s at 10,000g and then stored on ice until use. The enzyme preparation has been done three times each with 150 adult whiteflies.

AChE activity was measured by the method of Ellman et al. [29], in which the hydrolysis of the substrate analogue acetylthiocholine–iodide is measured colorimetrically by the absorbance of 2-nitro-5-thiobenzoate at 412 nm after the reaction of 5.5′-dithiobis-2-nitrobenzoate (DTNB) with the liberated thiocholine. Measurements were made at 25 °C with a final concentration of 1 mM acetylthiocholine iodide and 0.25 mM DTNB in 1 mL cuvettes in a spectrophotometer (Model 190 DES, Safas Monaco) in kinetic mode for 10 min. The amount of sample used was equivalent to 10 adult whiteflies to ensure a minimum of 0.2 OD after a reaction time of 10 min.

2.5. Inhibition kinetics

To measure the inhibition kinetics, 20 μL of sample was added to 70 μL of 25 mM phosphate buffer and 10 μL of the inhibitor (chlorpyriphos-oxon) solution or of distilled water (control). The mix was incubated at 25 °C for 2 min with chlorpyriphos-oxon. Remaining activity was recorded after 10 min after the addition of 900 μL Ellman solution containing 1 mM acetylthiocholine iodide and 0.25 mM DTNB. Each measurement was repeated 3 times.

2.6. Kinetic analyses

To follow the inhibition, the enzyme was incubated for times ranging from 2 to 10 min. The variation of the remaining non-inhibited enzyme with time was estimated by sampling aliquots at various times and recording the remaining activity with substrate. Disappearance of free enzyme (E) or disappearance of the inhibitor (I) follows pseudo-first order kinetics. After integration we used the following equation for determining the number of AChE variants in each population:

$$(E/Eo) = (A/Ao) = e^{(-ki \cdot Io \cdot t)} \quad (1)$$

where k_i is the pseudo-first order constant of inhibition, I_o is the inhibitor concentration, t is the incubation time of the enzyme with the inhibitor, E is the concentration of remaining free enzyme following incubation with the inhibitor, E_o is the concentration of free enzyme at time 0 and E/E_o is the remaining enzyme concentration, identical to remaining enzyme activity A/A_o.

2.7. Statistical analyses

Statistical analysis of toxicological and kinetics data was done using GOSA-fit available at http://bio-log.biz. This software permits the estimation of LD_{50}s of 2 sub-populations in a heterogeneous population. The fit was done on the equation of the sum of two Gauss cumulated curves. For kinetic analysis, the chi-square test was used in testing if a sample of data comes from a population described by a given distribution. A test statistic is calculated from the observed and calculated values according to the Eq. (2):

$$T = \sum \frac{(y_i - f(x_i))^2}{f(x_i)} \quad (2)$$

where x_i and y_i are the measured variables and $f(x_i)$ are values of the model function, computed on the basis of the expression. Given the confidence level and the number of degrees of freedom, a critical value T_{crit} can be computed, as well as the associated P-value, which constitute the reference for making decisions about the success or failure of the fit and comparison between different fits. Please note that in literature the chi-square test statistic is sometimes referred to as χ^2. We use a different symbol here (T) to avoid confusion with the goodness-of-fit parameter (when SD is associated with each data).

3. Results

3.1. Bioassay

LC_{50} of chlorpyriphos ethyl in a whitefly population collected from Bohicon (B4) was determined for both the hypothesis of a single population and the hypothesis of two sub-populations (Fig. 1). The fit of the one population hypothesis was compared to the fit of the two population hypothesis using the same slope. Statistically the two population model is better than the one population model (Chi-square test). In the two population model, the LC_{50} of the susceptible sub-population (0.18 ± 0.04 mg L^{-1}) was significantly lower than the LC_{50} of the resistant sub-population (10.72 ± 1.57 mg L^{-1}) with the same slope of 2.8 ± 0.5. The percentage of susceptible individuals in this population was estimated to be 52.1 ± 3.5%.

3.2. Biochemical assays

3.2.1. Determination of the number of AChEs with different sensitivity to chlorpyriphos in susceptible strain and in one field population

Inhibition of AChE activity in whiteflies from the susceptible Sud-S strain (B1) and in the Ben-Pah field population (B3) with chlorpyriphos concentrations was analyzed for the possibilities that there is only one enzyme (Fig. 2 and 3A), two enzymes (Fig. 2 and 3B) or three enzymes (Fig. 2 and 3C). For one enzyme, the fit using Eq. (1) was not satisfactory for both populations. Then the presence of two different proteins was considered. In that case, the Eq. (3) was used with a as the proportion of one enzyme activity inhibited with the constant of inhibition $ki1$ and $(1 - a)$ as the proportion of the second enzyme activity inhibited with the constant of inhibition $ki2$ and with $ki1 > ki2$:

$$(A/Ao) = a.e^{(-ki1.Io.t)} + (1 - a).e^{(-ki2.Io.t)} \quad (3)$$

Increasing the number of parameters significantly increased the fit, i.e. diminished the sum of squared deviations and was satisfactory. Thus curve was better explained considering that there were two enzymes in the sample compared with one. To test the presence of three enzymes, the equation was enlarged to three ki and two coefficients (a and b). In that case, the Eq. (4) was used with a as the proportion of one enzyme activity inhibited with the constant of inhibition $ki1$, b as the proportion of one enzyme activity inhibited with the constant of inhibition $ki2$ and $(1 - a - b)$ as the proportion of the third enzyme activity inhibited with the constant of inhibition $ki3$ and with $ki1 > ki2 > ki3$:

$$(A/Ao) = a \cdot e^{(-ki1.Io.t)} + b \cdot e^{(-ki2.Io.t)} + (1 - a - b) \cdot e^{(-ki3.Io.t)} \quad (4)$$

In that case the difference between the models was statistically significant for Ben-Pah field population (B3) but not for Sud-S susceptible strain. For the field population B3 the model with three enzymes was superior to the model with twp enzymes (Chi-square test). The fit was significantly increased. Consequently we can conclude that there are two AChEs in the susceptible strain and three AChEs in the field population. With two AChEs, the sensitive AChE from Sud-S strain was inhibited by chlorpyriphos at the rate $ki1$ = 0.70 ± 0.24 μM^{-1} min^{-1}, when the less sensitive one was inhibited at rate $ki2$ = 0.02 ± 0.05 μM^{-1} min^{-1}.

3.2.2. Determination of the number of AChEs with different sensitivity to chlorpyriphos in 7 populations

To show evidence for three enzymes, we enlarged the analysis to seven populations (Bj). The analysis was made with Eqs. (5) and (6) which considered two and three enzymes, respectively. As the kis are characteristic of the enzyme, the only parameters expected to vary were the proportion of AChEs activity in the different populations:

$$(A/Ao)_{Bj} = a_{Bj}.e^{(-ki1.Io.t)} + (1 - a_{Bj}).e^{(-ki2.Io.t)} \quad (5)$$

$$(A/Ao)_{Bj} = a_{Bj}.e^{(-ki1.Io.t)} + b_{Bj}.e^{(-ki2.Io.t)} + (1 - a_{Bj} - b_{Bj}).e^{(-ki3.Io.t)} \quad (6)$$

The simultaneous fit of data to equations was made for two (Fig. 4A) and three (Fig. 4B) enzymes. The model with three enzymes was superior when compared with that for two (Chi-square

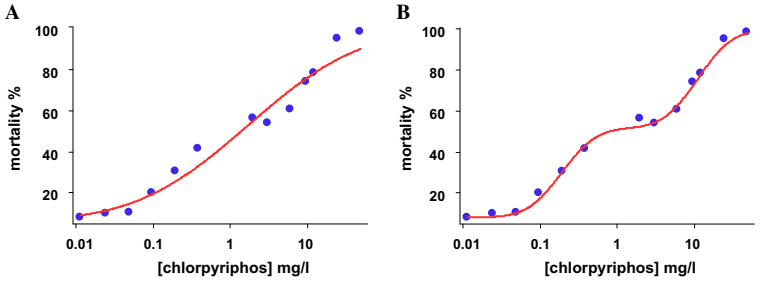

Fig. 1. Toxicity of chlorpyriphos ethyl on *B. tabaci* from the field population Ben-Boh (B4). On the left (A), the fit was done with one population hypothesis using the equation of a Gauss cumulated curve (LC_{50} = 1.66 ± 0.86 mg a.i. L^{-1}; slope = 0.87 ± 0.21; χ^2 = 8.7). On the right (B), the fit was done with two sub-populations hypothesis (S & R) using the equation of the sum of two Gauss cumulated curves with the same slope (LC_{50} S = 0.18 ± 0.04 mg a.i. L^{-1}; LC_{50} R = 10.72 ± 1.57 mg a.i. L^{-1}; slope = 2.8 ± 0.53; χ^2 = 1.3).

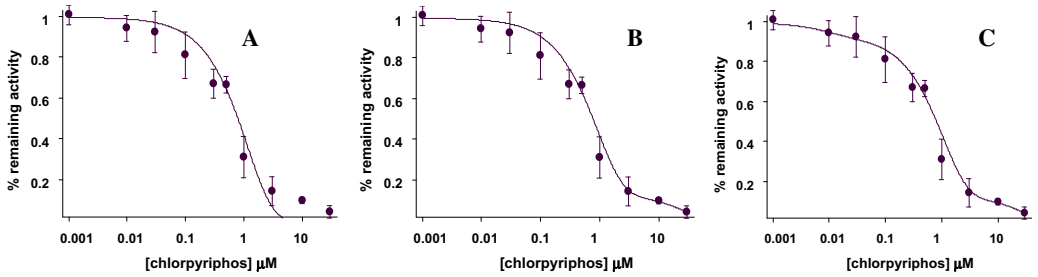

Fig. 2. Inhibition of AChE activity in *B. tabaci* from the strain SUD-S (B1) by chlorpyriphos oxon. The fit is done with one enzyme hypothesis and fit is not optimal (*P*-value < 0.05) (A); The fit is done with two enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (B); The fit is done with three enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (C). The difference between the models 2 AChE and 3 AChE was not statistically significant.

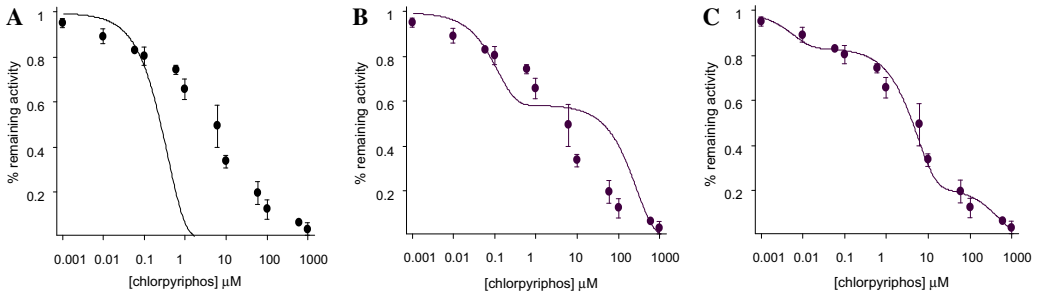

Fig. 3. Inhibition of AChE activity in *B. tabaci* from the field strain Ben-Pah (B3) by chlorpyriphos oxon. The fit is done with one enzyme hypothesis and fit is not optimal (*P*-value < 0.05) (A); The fit is done with two enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (B); The fit is done with three enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (C). The difference between the models 2 AChE and 3 AChE is statistically significant. The 3 AChE model is superior (lower sum of squared deviations).

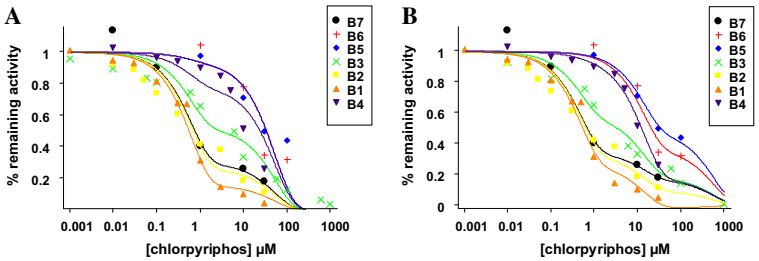

Fig. 4. Inhibition of AChE activity by chlorpyriphos oxon in *B. tabaci* from seven strains: Sud-S (B1), Ben-Ago (B2), Ben-Pah (B3), Ben-Boh (B4), Arizona University (B5), Mexico S2 (B6) and UK (B7). The fit is done with one enzyme hypothesis and fit is not optimal (*P*-value < 0.05) (A); The fit is done with two enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (B); The fit is done with three enzymes hypothesis and fit passes the chi-square test (*P*-value > 0.05) (C). The difference between the models 2 AChE and 3 AChE is statistically significant. The 3 AChE model is superior (lower sum of squared deviations).

test). Consequently we can confirm that there are three AChEs. Considering there are three enzymes, the sensitive AChE was inhibited by chlorpyriphos at the rate $ki1 = 1.193 \pm 0.438\ \mu M^{-1}\ min^{-1}$, when the less sensitive one was inhibited at rate $ki3 = 0.001 \pm 0.001\ \mu M^{-1}\ min^{-1}$ and the intermediate was inhibited at rate $ki2 = 0.035 \pm 0.019\ \mu M^{-1}\ min^{-1}$. More precision has obviously been obtained for *kis* with 7 populations compared with those obtained with one population. The populations were distributed in three groups (Fig. 4B): a susceptible group with the B1, B2 and B7 populations where a low dose of insecticide inhibited 50% of AChE, a less sensitive group with the B4, B5 and B6 populations where a higher concentration of insecticide was used for inhibiting 50% of AChE.

3.2.3. Activity of the different AChE

Proportions of AChEs activity inhibited by insecticide in each of population have been obtained (Table 1). It appears that activity decreases with the proportion of sensitive enzyme, thus insensitive enzymes are less active than the sensitive enzyme. In the reference strain, B1, $AChE1_S$ is responsible for 69% of the total activity and

Table 1
Estimation of proportion of AChE variants in seven *Bemisia tabaci* strains: susceptible (AChE1S), resistant (AChE1R) and non synaptic (AChE2).

Strains	Code	AChET (nmol)[a]	$AChE1_S$/AChET[b]	$AChE1_R$/AChET[c]	AChE2/AChET[d]	AChE1S (nmol/min/ind.)	AChE1R (nmol/min/ind.)	AChE2 (nmol/min/ind.)
Sud-S	B1	0.38	0.69 ± 0.13	0	0.31 ± 0.24	0.26	0	0.12
Ben-Ago	B2	0.24	0.65 ± 0.13	0.1	0.25 ± 0.23	0.16	0.02	0.06
Ben-Pah	B3	0.10	0.37 ± 0.12	0.16	0.47 ± 0.18	0.04	0.02	0.05
Ben-Boh	B4	0.13	0.04 ± 0.11	0.15	0.81 ± 0.25	0.01	0.02	0.10
Arizona Univ.	B5	0.13	0 ± 0.15	0.47	0.53 ± 0.23	0	0.06	0.07
Mexico-S2	B6	0.15	0 ± 0.26	0.35	0.65 ± 0.31	0	0.05	0.10
UK	B7	0.41	0.63 ± 0.16	0.16	0.21 ± 0.26	0.26	0.07	0.09

[a] Total activity in nmol of substrate hydrolyzed per minute and per insect.
[b] Proportion of susceptible AChE activity in the total activity.
[c] Proportion of resistant AChE activity in the total activity (c = 1 − b − d).
[d] Proportion of non synaptic AChE activity in the total activity.

therefore has an activity of 0.26 nmol min^{-1} $insect^{-1}$ with a *ki1* = 1.193 ± 0.438 μM^{-1} min^{-1}. There is no resistant $AChE1_R$. The resistant AChE comes only from the activity of AChE2 which is responsible for 31% of the total activity, thus AChE2 has an activity of 0.12 nmol min^{-1} $insect^{-1}$ with a *ki2* = 0.035 ± 0.019 μM^{-1} min^{-1}. When there is practically no susceptible enzyme, as in the B4, B5, and B6 populations, the total activity is due partly to $AChE1_R$ and partly to AChE2. Considering that AChE2 does not vary with resistance, its average activity is 0.08 ± 0.02 nmol min^{-1} $insect^{-1}$. Thus $AChE1_R$ has an inhibition rate *ki3* = 0.001 ± 0.001 μM^{-1} min^{-1} and an activity about 13-fold lower than the activity of $AChE1_S$ in the referent strain SUD-S (B1) in the experimental conditions used (1 mM acetylthiocholine, pH 7).

4. Discussion

In the present study, we were able to show evidence for chlorpyriphos resistant and susceptible sub-populations in the Benin field population. The LC_{50} of the resistant field sub-population was significantly higher than the LC_{50} of the susceptible SUD-S reported in literature with quite the same slope confirming the chlorpyriphos resistance [26]. This method appeared useful for analysing the toxicological effect of an insecticide on a heterogeneous population without any reference strain. But it needs many toxicological data from each sub-population. In the reference strain SUD-S two different acetylcholinesterases were detected with different sensitivity to chlorpyriphos oxon suggesting the presence of two genes ace 1 and ace 2. In field populations, in addition to ace 2, two alleles may be detected at the same gene locus ace 1: one susceptible ace1S and one resistant ace1R. As SUD-S (B1) is the reference susceptible strain, there are two AChEs: one responsible for the synaptic transmission and sensitive to OPs (AChE1) and one not involved in the nerve impulse transmission (AChE2) and less sensitive to OPs. In addition, we consider that AChE1 has two forms one sensitive ($AChE1_S$) and one resistant ($AChE1_R$). We know that resistance to organophosphorus insecticide is mainly due to insensitivity of the target enzyme AChE. Also carboxylesterases could play a role as reported for *B. tabaci* from Israel [26]. But first studies of chlorpyriphos toxicity using pretreatment with an esterase inhibitor (DEF) did not showed any synergistic effect (unpublished data). There are two AChE genes in most insects, one encoding a synaptic protein which is involved in insecticide toxicity (ace1) and another one encoding a non synaptic protein (ace2), not involved in the nerve influx transmission [30–34]. These two proteins, synaptic (AChE1) and non synaptic (AChE2), can be distinguished, each with their own substrate specificity and kinetic parameters. Bourguet et al. [30] characterized them by their molecular forms in *Culex pipiens* with a discriminating dose of propoxur present in the staining solution and shown that AChE1 was inhibited and AChE2 was not inhibited. These authors concluded that only AChE1 is involved in nerve influx transmission and the resistance to insecticides observed in mosquito larvae is due to the AChE1 modification. In our study, we found that AChE1 is 3-fold more sensitive to chlorpyriphos-oxon than AChE2.

Modified types of AChE have already been reported in whitefly. Byrne et al. [24] have found in Israeli field population of whitefly, two AChE variants different in their response to paraoxon. In 2002, Byrne and Toscano [7] distinguished sensitive and insensitive AChE variants in whitefly using chlorpyriphos-oxon and sulprofos with meta-chloroperoxybenzoic acid. These results could be explained by a modification in AChE1 where a mutation changed the sensitive molecule ($AChE1_S$) in a resistant one ($AChE1_R$). We found three AChE where $AChE1_R$ has 5-fold less activity than $AChE1_S$ with an insensitivity ratio about 1000 determined by dividing *ki* of $AChE1_S$ by *ki* of $AChE1_R$. In our study we tested chlorpyriphos-oxon on various populations and found evidence for the three forms of AChE. In fact, these three forms were not demonstrated until we used chlorpyriphos-oxon on the B3 population which had an intermediate proportion of $AChE1_S$ (0.37) compared with B4 (0.04) and B2 (0.65). Then, the simultaneous fit of data of the seven populations confirmed the presence of three AChE, providing more certainty than the result obtained with one population alone. Based on the studies made by Alon et al. [26] where the comparison of ace1 coding sequence between the OP-resistant strain B-ref and the OP-susceptible strain SUD-S revealed one amino acid substitution in B-ref from phenylalanine to tryptophan at position 392 (Phe392Trp) and silent nucleotide substitution in SUD-S and B-ref of the ace2, it is possible to have in West Africa, populations of *B. tabaci* in which resistance to chlorpyriphos is associated with the ace1 type.

In southern Benin field populations there was a high level of variation in the proportion of $AChE1_S$ (from 4% to 65%). The proportion of $AChE1_R$ in B4 population collected on cotton was higher than for the populations B2 and B3 collected on eggplant and mint, respectively. This result could be explained by the greater use of organophosphorus insecticides on cotton in West Africa for over 40 years [9] and their rare use on indigenous vegetables but not on mint where insects were collected. The reduced total AChE1 activity in resistant individual may be responsible for a fitness cost since decrease in AChE activity is known to alter development in insects [35]. Thus, AChE resistance could be expected to decrease in non-treated areas, due to interbreeding and the competition between resistant and susceptible whiteflies. AChE resistance mechanism in *B. tabaci* populations from Benin may explain why OP insecticides used on cotton against whiteflies such as dimethoate and omethoate are become inefficient and have been recently replaced by neonicotinoids such as acetamiprid. But chlorpyriphos in mixture with pyrethroid insecticides is still largely used on cotton against leafworms and mites maintaining the selection

pressure on *B. tabaci*. As this mixture, bought in large quantity for cotton farmers by ginning companies, is also largely used on vegetables against various pests including whiteflies [36], there is a risk of increase chlorpyriphos resistance level in *B. tabaci* populations increasing an overuse of insecticide by small-scale farmers. The identification of point mutation occurred in the African populations of *B. tabaci* [26,37] responsible of AChE resistance has to be the next step. Then the frequency of AChE resistant allele in *B. tabaci* populations could be surveyed as an indicator to evaluate the insecticide selection pressure with OPs and carbamates in farmer fields for managing insecticide resistance [37].

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An acaricide-treated net to control phytophagous mites

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ABSTRACT

A new technique to control phytophagous mites was tested in West Africa on the African eggplant *Solanum macrocarpon*. This technique consisted of covering plants with a net impregnated with the acaricide dicofol. The net was applied during the night to protect the net fabric and the active ingredient from UV degradation and to avoid interference of the net with overhead watering. The technique was evaluated in three trials at the INRAB research station and in a grower's field in 2006 and 2007. Results showed that the broad mite *Polyphagotarsonemus latus* (Banks), the major pest of *S. macrocarpon* which causes severe damage to leaves, and spider mites (*Tetranychus* spp.), were completely controlled by the dicofol-treated net. Very few mites and practically no mite injury were observed on plants covered with the dicofol-treated net compared to 12–94% damaged leaves in the unprotected control plots. The acaricide-treated net was as efficient when used temporarily (once every three nights) as when used every night. No difference in the percentages of leaves damaged by mites was apparent at harvest between plots covered with a non-acaricide deltamethrin-treated net (40%) and unprotected control (32%). This new concept of mite control using an acaricide-treated net temporarily covering vegetable crop appears to be an efficient tool which is easy to use. It can be used repeatedly reducing costs and poses a very low risk of environmental pollution when used in the dry season or in greenhouses.

1. Introduction

The African eggplant *Solanum macrocarpon* L., locally named gboma, is the most important indigenous leafy vegetable grown in urban and peri-urban areas in Benin, West Africa. It is susceptible to different species of leaf feeding arthropods, the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) being the major economic pest especially in the southern part of the country (Adango et al., 2006). Additionally, two other phytophagous mites in the family Tetranychidae, *Tetranychus urticae* Koch and *Tetranychus ludeni* Zacher might be a serious problem. Broad mites feed by piercing plant cells and sucking the sap that leaks from the wound (Gerson, 1992). Photosynthesis is reduced in the plant, and water balance is affected. The apical leaves are usually heavily damaged, grow distorted and often die. These symptoms persist for weeks, suggesting damage by mite toxins. In southern Benin, growers perceive broad mite damage as one of the most important constraints to *S. macrocarpon* production. In a study conducted in 10 vegetable production sites, up to 90% of *S. macrocarpon* plants were damaged by mites (James et al., 2005). According to the authors, the frequency of application of pesticide on *S. macrocarpon* reported by 50 growers was about once a week with on average 7 insecticide applications of deltamethrin or bifenthrin. Such insecticide overuse could be explained by the ineffectiveness of pyrethroids to control broad mites (Vaissayre, 1986) whilst adversely affecting their predators (i.e. Phytoseiidae) and maybe the lack of efficient acaricidal products available in the country.

Integrated Pest Management (IPM) can be a viable alternative, including use of chemicals, host plant resistance and biological control. Chemical control gives variable results in controlling mites due to the difficulties of applying the spray to the underside of leaves and to the selection of resistance within spider mite populations (Cranham and Helle, 1985; Nauen et al., 2001). But foliar sprays with pesticides frequently used by small-scale farmers pose a high risk of operator contamination for users and pollute the environment including groundwater. Biological control with predatory mites has been proven effective in controlling mites on various vegetable crops particularly in greenhouses (Gerson, 1992; Peña et al., 1996; Weintraub et al., 2003) and sometimes in the field

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Table 1
Percentage of damaged plants and apical leaves by mites during the vegetative stage and all damaged leaves at harvest with the yield of *Solanum macrocarpon* protected with a dicofol or a deltamethrin-treated net used every night compared with an unprotected control in the first trial at the INRAB research station in 2006.

Treatments	Damaged plants during vegetative stage[a]		Damaged apical leaves during vegetative stage[b]		Damaged leaves at harvest		Yield of leaves	
	%	95%CI	%	95%CI	%	95%CI	kg/100 m^{-2}	95%CI
Dicofol-treated net	1.3 a	±0.9	0.0 a	±0.0	5.6 a	±1.5	180 a	±57
Deltamethrin-treated net	14.2 b	±2.5	14.4 c	±3.2	40.5 b	±8.2	105 b	±24
Unprotected control	16.1 b	±4.0	7.0 b	±3.9	32.0 b	±18.6	209 a	±48

Note: means in the same column with the same letters are not significantly different ($P < 0.05$).
[a] Average on 11 sampling dates twice a week (90 plants observed per treatment and per date).
[b] Average of 11 sampling dates twice a week (450 leaves observed per treatment and per date).

(Reddy, 2001). But predatory mites are not easy to use for many small-scale farmers who cultivate very small plots intensively in the same urban or peri-urban area.

Taking into account the efficiency of a deltamethrin-treated net in controlling the aphid *Lipaphis erysimi* (Kaltenbach) on cabbage (Martin et al., 2006; Licciardi et al., 2008) and the efficiency of the acaricide dicofol in controlling *P. latus* and *T. urticae* in Africa (Vaissayre, 1986), the present study was initiated in Benin to determine the effectiveness of an acaricide-treated net to control mites on *S. macrocarpon*. A dicofol-treated net was initially tested in 2006 at the research station where *S. macrocarpon* was covered every night compared with a deltamethrin-treated net and an unprotected control. To confirm and complete these results, two trials were implemented the following year at the research station and in a grower's field where the dicofol-treated net was used once every three nights versus every night, compared with an untreated net used every night and an unprotected control.

2. Materials and methods

2.1. Experimental design

The first trial was carried out at the Agricultural Research Station of the Institut National de Recherche Agronomique du Bénin (INRAB), in Abomey Calavi, from January to April 2006. A local variety of *S. macrocarpon* was sown in a nursery. After 20 days, six rows of the eggplants were planted out in 9 plots of 4.5 m^2 (1.5 m × 3 m). Three treatments were replicated three times and randomly assigned in an experimental design. The treatments consisted of a dicofol-treated net covering the plant every night, a deltamethrin-treated net covering the plant every night and an unprotected control. Harvest was done 7 weeks after planting.

The second trial was carried out the following year in the same research station from January to April 2007. After 30 days in the nursery, five rows of *S. macrocarpon* were planted in 24 plots of 6 m^2 (1 m × 6 m). Four treatments were replicated six times and randomly assigned to plots in a randomised complete block design. The treatments consisted of a dicofol-treated net covering the plots every night, a dicofol-treated net covering the plots once every three nights; an untreated net covering the plots every night, and an unprotected control. Harvest was done 7 weeks after planting.

The third trial was carried out in the same period in a growing urban area of Cotonou. Five rows of *S. macrocarpon* were planted out in 9 plots of 6 m^2 (1 m × 6 m). Three treatments were replicated 3 times in 3 blocks. The treatments consisted of a dicofol-treated net used every night; a dicofol-treated net used once every three nights, and an unprotected control. Harvest was done 8 weeks after planting.

2.2. Nets

Nets, obtained from the local market, were simple white netting made of knitted polyester, weighting 30 g/m^2 and with 40 holes/cm^2. Six nets were cut to an appropriate size to cover largely the plants (2 m × 4 m for the first trial and 2 m × 7 m for the second

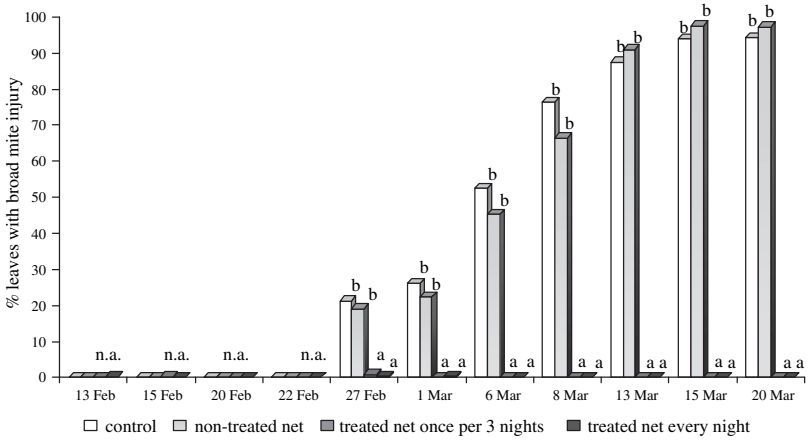

Fig. 1. Temporal evolution of damage leaves by broad mites on *Solanum macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an untreated net and an unprotected control in the second trial at the INRAB research station in 2007. Means at the same sampling date with the same superscript are not significantly different ($P < 0.05$). n.a.: non-analysed.

Table 2
Percentage of damaged leaves by mites during the vegetative stage (5 apical leaves) and at harvest (all leaves) with the yield of *S. macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an untreated net and an unprotected control in the second trial at the INRAB research station in 2007.

Treatments	Damaged apical leaves during vegetative stage[a]		Damaged leaves at harvest		Yield of leaves	
	%	95%CI	%	95%CI	kg/100 m^{-2}	95%CI
Dicofol-treated net once per 3 nights	0.1 a	±0.0	0.0 a	±0.0	1939	±973
Dicofol-treated net every night	0.1 a	±0.0	0.0 a	±0.0	1955	±1021
Untreated net every night	62.7 b	±21.3	95.9 b	±5.2	1640	±589
Unprotected control	64.6 b	±22.0	97.2 b	±3.2	1635	±489

Note: means in the same column with the same letters are not significantly different ($P < 0.05$).
[a] Average of seven sampling dates twice a week (900 leaves observed per treatment and per date).

and third trials). The nets were placed directly on the plants in contact with apical leaves when they were sufficiently robust. Nets were placed at 5 p.m. and removed the following morning at 9 a.m. For plots protected with a dicofol-treated net once every three nights in the second and third trial, one net was used for three replications with a daily rotation. Thus for this treatment, 1 of 3 (in the grower's field) or 2 of 6 (at the research station) replicated plots were protected each night with the dicofol-treated net. Vinyl gloves were used to handle the nets.

2.3. Laboratory procedure for the pesticide treatment of polyester nets

For treatment of one entire 14 m^2 net (2 m × 7 m) at a target dose of 50 mg deltamethrin active ingredient per square metre (a.i. m^{-2}) or at a target dose of 50 mg dicofol a.i. m^{-2}, deltamethrin 12.5 EC from ALM (Abidjan, Côte d'Ivoire) and Kelthane 45 EC from Hydrochem (Cotonou, Benin) were used, respectively. The treated nets were impregnated once before the trials to obtain a final rate of 50 mg a.i. m^{-2}, taking into account the net absorption after soaking up (54.75 ml m^{-2}) and the concentration of the formulation. Thus for treating a 14 m^2 dicofol-treated net, the polyester net was dipped in a bowl of suitable size containing 15.6 ml of Keltane 45 EC (45 g dicofol a.i. l^{-1}) with 751 ml of water. The net was kneaded with gloved hands for 5 min until it was thoroughly and evenly soaked and until the mixture was completely absorbed by the net. The net was then taken out of the bowl, unfolded and put flat on the floor to dry at ambient temperature (28 °C). After 1 h, the net was then hung over a laundry line to dry for another 24 h until completely dry.

2.4. Sampling

The number of plants with broad mite injury was counted on 30 plants per plot, twice a week at the research station and once a week in the farmer's fields. The total number of plants per plot was about 180 for the first experiment and about 240 for the second and the third. The number of apical leaves with broad mite injury was counted on the five terminal leaves of 30 plants per plot twice a week at the research station and once a week in the farmer's fields. The mites *P. latus* and *Tetranychus* spp., were counted once a week on the third apical leaves from 10 plants per plot, separating motile stages and eggs. Ten leaves per plot (one per plant) were cut off for examination the same day in the laboratory with a binocular microscope. Harvest was done 7–8 weeks after planting on the four (trial 1) or the three (trials 2 and 3) middle rows of the plots. The number and the weight of healthy leaves were recorded, not counting unmarketable damaged leaves. Leaves damaged were considered as "unmarketable" when they were heavily damaged and distorted. For local people these kinds of leaves are hard and not tasty.

2.5. Analysis

Minitab® software was used for statistical analysis. Biological data such as mite counts and percentages of infested leaves were compared between groups using the Mann–Whitney non-parametric test. Zero data from sampling dates before the beginning of mite infestation were not been taken into account in averages. Analysis of variance was used for harvest data analysis with a Tukey test ($P < 0.05$) to separate means.

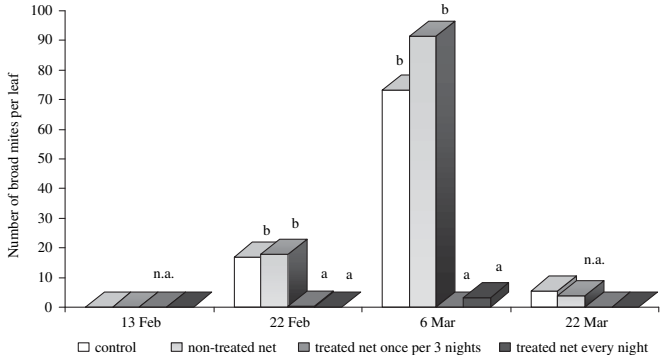

Fig. 2. Temporal evolution of broad mites (number of motile stage per leaf) on *S. macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an untreated net and an unprotected control in the second trial at the INRAB research station in 2007. Means at the same sampling date with the same letters are not significantly different ($P < 0.05$). n.a.: non-analysed.

Table 3
Mean number per leaf of *P. latus* and *Tetranychus* spp. on *S. macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an untreated net and an unprotected control in the second trial at the INRAB research station in 2007.

Treatments	*P. latus*				*Tetranychus* spp.			
	Eggs	95%CI	Motile stages	95%CI	Eggs	95%CI	Motile stages	95%CI
Dicofol-treated net once per 3 nights	0.1 a	±0.2	0.4 a	±0.6	0.0	±0.0	0.0	±0.0
Dicofol-treated net every night	3.0 a	±5.0	3.2 a	±4.8	0.2	±0.3	0.1	±0.1
Untreated net every night	181.9 b	±42.3	113.0 b	±31.8	0.4	±0.6	0.4	±0.7
Unprotected control	140.3 b	±27.9	95.3 b	±15.1	0.9	±1.2	0.8	±0.8

Note: means in the same column with the same letter are not significantly different ($P < 0.05$).
Cumulated data of 3 sampling dates once a week (60 leaves observed per treatment and per date).

3. Results

The major pest observed on *S. macrocarpon* in the three trials implemented in the dry season was the broad mite *P. latus*. The spider mites *Tetranychus* spp were observed at low densities. The mite infestation always increased from planting to harvest. Apical leaves were the most heavily infested leaves with mean numbers of motile stages sometimes >100 per leaf. At harvest time, 10–95% of leaves were damaged by mites. Leaf eating pests such as the grasshopper *Zonocerus variegatus* (L.) and the caterpillar *Selepa docilis* (Butler) were rarely observed.

3.1. Trial in the research station in 2006

The protection of *S. macrocarpon* against mites obtained with the dicofol-treated net was very efficient compared to the other treatments (Table 1). During the vegetative stage the percentage of plants and apical leaves damaged by mites were significantly lower than other treatments. The effectiveness of dicofol-treated nets was confirmed by lower leaves damaged by mites at harvest, compared with 32% of total leaves damaged for the unprotected control. The worst results were obtained with the deltamethrin-treated net (40.5% damaged leaves in average). The percentage of apical leaves damaged during the vegetative stage and the percentage of leaves damaged at harvest were significantly higher than the unprotected control (Table 1). Moreover the yield of leaves was significantly lower with the deltamethrin-treated net than with the other treatments. Mite populations were not recorded in this first trial because the effect of this technique to protect African eggplant against mites was not known at the time. As recording mite population is very labour intensive, the observations were limited to mite damages. After having noted the good efficacy of a dicofol-treated net to prevent broad mite damages, mite populations were recorded in the following trials.

3.2. Trial in the research station in 2007

The efficiency of the dicofol-treated net to protect *S. macrocarpon* against mite injuries was confirmed in an area with a high infestation of mites. There were practically no apical leaves damaged by mites with the dicofol-treated net compared to an average of 65% of damaged apical leaves with the unprotected control at the vegetative stage and reaching 97% of damaged leaves at harvest time (Fig. 1, Table 2). The dicofol-treated net was particularly efficient in controlling *P. latus* (Fig. 2). There were practically no eggs and motile stages with the dicofol-treated net compared with about 140 eggs and 95 motile stages per leaf in the unprotected control (Table 3). There was no significant difference between treatments for *Tetranychus* spp. which were rarely observed in this trial. The use of dicofol-treated nets once every three nights was as effective as used every night as there was no significant difference between the two treatments for the variables observed (Tables 2 and 3). The untreated net had no effect on mite control as there was no significant difference with the unprotected control for the variables observed (Tables 2 and 3). At harvest time, there was no significant difference in the yield of leaves between treatments. Meanwhile there was no leaf damage on *S. macrocarpon* protected by dicofol-treated nets while practically all leaves with the untreated nets and in the unprotected control suffered mite injuries (Table 2).

3.3. Trial in a grower's field in 2007

The effectiveness of the dicofol-treated net in controlling mites on *S. macrocarpon* was also confirmed in this trial. There were practically no damaged apical leaves with the dicofol-treated net used once every three nights or every night during the vegetative stage compared with 38% on average in the unprotected control (Table 4). At harvest, the yield of leaves was significantly higher with the dicofol-treated nets with no damaged leaves compared with 11% of damaged leaves for the unprotected control. The efficacy of the dicofol-treated nets to control *P. latus* was confirmed with very few motile stages observed compared with about 209 motile stages per leaf in the unprotected control (Table 5). Moreover the number of *Tetranychus* spp. was significantly lower with the dicofol-treated nets than the unprotected control, demonstrating the effect of this technique to control these species of mites.

Table 4
Trial in percentage of damaged leaves by mites during the vegetative stage (5 apical leaves) and at harvest time (all leaves) with the yield of *S. macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an unprotected control in the trial at a grower's field in 2007.

Treatments	Damaged apical leaves during vegetative stage[a]		Damaged leaves at harvest		Yield of leaves	
	%	95%CI	%	95%CI	kg/100 m^{-2}	95%CI
Dicofol-treated net once per 3 nights	0.5 a	±0.5	0.0 a	±0	1351 a	±18
Dicofol-treated net every night	0.7 a	±0.5	0.0 a	±0	1283 a	±201
Unprotected control	38.0 b	±5.0	11.0 b	±4.8	793 b	±133

Note: Means in the same column with the same letter are not significantly different ($P < 0.05$).
[a] Average of three sampling dates once a week (450 leaves observed per treatment and per date).

Table 5
Mean number per leaf of *P. latus* and *Tetranychus spp* on *S. macrocarpon* protected with a dicofol-treated net used once every three nights or every night compared with an unprotected control in the trial at a grower's field in 2007.

Treatments	*P. latus*		*Tetranychus* spp.	
	Motile stages	95%CI	Motile stages	95%CI
Dicofol-treated net once per 3 nights	2.8 a	±3.7	0.2 a	±0.3
Dicofol-treated net every night	7.3 a	±9.4	0.1 a	±0.1
Unprotected control	208.8 b	±105.7	4.5 b	±1.1

Note: Means in the same column with the same letter are not significantly different ($P < 0.05$).
Cumulated data of 3 sampling dates once a week (30 leaves observed per treatment and per date).

4. Discussion

The acaricide-treated net was particularly effective in controlling phytophagous mites. On *S. macrocarpon*, the dicofol-treated net seemed to control *P. latus* as well as *Tetranychus* spp. since there were very few mites and practically no injury symptoms due to *P. latus* on leaves contrary to the unprotected control and despite heavy infestations of the mite throughout the season. When *S. macrocarpon* were protected with a dicofol-treated net every night or twice a week from the beginning of mite infestation until two weeks before harvest, the yields in weights of leaves were generally higher than the unprotected control, and the leaves did not present any apparent broad mite injury. Broad mites cause terminal leaves to become malformed because toxic saliva causes twisted, hardened and distorted growth including in the bottom of the plant (Gerson, 1992). Without any protection against mites more than 90% of leaves of *S. macrocarpon* could appear with broad mite injuries devaluing the quality of production. Moreover, the protection with the dicofol-treated net was fully effective both at levels of high (unpublished data) and low densities of mite, which may be important if, as Bassett (1981) has suggested, only a few mites suffice to invoke economic damage on certain hosts.

The effectiveness of the acaricide-treated net in controlling mites was due to the impregnation of the net with the acaricide dicofol in contrast to a net impregnated with the non-acaricide deltamethrin or an untreated net which had no effect on mite control. Mites can reach un-infested hosts by wind dispersal (Aubert et al., 1981) or by attaching themselves to winged whitefly adults as reported by Parker and Gerson (1994) and Palevsky et al. (2001). On the plant, Pralavorio et al. (1989) showed an alternate vertical movement of high populations of the mite *T. urticae* up and down plant stems. Thus we hypothesise that mites use the net during their circadian migration receiving a toxic dose of dicofol when walking on the treated net. This hypothesis was confirmed by the dicofol-treated net which remained efficient when covering over the plants only once every three nights. Such mite dispersal has been reported by Smith (1935) who found that mites 'crossed' (presumably by walking) an 18-inch space between plants. The broad mite is usually found on the lower surfaces of young apical leaves where its eggs are deposited (Gerson, 1992). *P. latus* moves upwards on individual plants, with the male carrying a female on his back, and 'chooses' the female oviposition sites (Gadd, 1946; Adango et al., 2006). But the findings of Pralavorio et al. (1989) indicated a migration of *T. urticae* to the top of the plants during the day and a migration to the bottom of the plants during night. *P. latus* circadian migration has not been studied. Thus the comportment of these mites in tropical conditions needs further investigations to explain the efficacy of this technique. The results showed highest mite infestations with the deltamethrin-treated net confirming the inefficacy of this pyrethroid to control mites (Vaissayre, 1986). Some pyrethroids might actually improve the fecundity of spider mite females, shorten development duration and bias the female:male ratio in favour of the former (Iftner and Hall, 1984; Watson et al., 1985; Gerson and Cohen, 1989) but there is no evidence that SPs stimulate broad mite development. Thus taking into account the ineffectiveness of SPs against broad mites (Vaissayre, 1986), consequent higher populations observed may well have resulted from SP-induced decreases of their predators.

To protect the netting and the dicofol from UV degradation, the acaricide-treated net was used during the night covering the *S. macrocarpon* plants between 5 pm and 9 am. The last results showed that it only needs to be used once every three nights for a very efficient control of phytophagous mites. Therefore we may suppose that an acaricide-treated net could be used in conjunction with an infestation threshold in a similar way to a foliar spray, but reusable many times with a very low risk of environmental contamination by residues. Dicofol is an organochlorine compound moderately hazardous with a toxicity class III (The e-Pesticide Manual 2.0®). To reduce the risk of human and environmental contamination, this new mite control technique could benefit from the development of fabric treatment technology that produces longer-lasting insecticidal treated materials for use against malaria vectors (Greenwood et al., 2005). For example, the long-lasting insecticidal nets are factory pre-treated and retain their biological effectiveness for at least 20 standard washes under laboratory conditions and three years of use under field conditions (WHO, 2005). In this case, a very small quantity of active ingredient is present on the outer surface of the material, the large part being incorporated into the fibres of the net (e.g. Olyset®) or incorporated in a polymer binder (e.g. Permanet®, K-O TAB®).

Dicofol is a non-systemic acaricide with little insecticidal activity which is recommended for control of many species of phytophagous mites on a wide range of crops, including vegetables, fruit, vines, ornamentals and field crops (The e-Pesticide Manual 2.0®). Thus a dicofol-treated net should have a lower effect on beneficial insects such as the most effective generalist insect predator *Chrysoperla carnea* Stephens (New, 1975). Meanwhile potential negative effects of acaricide-treated nets on predatory mites need to be studied. The release of *Neoseiulus cucumeris* (Oudemans) in greenhouse is known to be very effective at controlling moderate levels of mites and thrips (Fan and Petitt, 1994; Peña et al., 1996; Weintraub et al., 2003). In this case and also for resistance problems as reported for spider mites (Dennehy and Granett, 1984; Cranham and Helle, 1985; Pree, 1987), the acaricide-treated net could be impregnated with other acaricidal products. For instance, bifenazate has a high toxicity when applied topically on all life stages of *T. urticae*, no cross-resistance was found to mites resistant to several classes of acaricides and seemed to have no effect on two predatory mites (Ochiai et al., 2007). Thus, additional studies are needed to investigate different types of acaricide-treated nets used in mosaics or in alternation to control resistant mites with various active ingredients on various plants and against various mite species, in combination with biological control methods. In greenhouses, particularly where populations of mites can increase rapidly on vegetables or ornamental flowers (Blindeman and Van Labeke, 2003; Ay et al., 2005), acaricide-treated nets could be used to control outbreaks just before the prompt release of

predators as is permitted by the absence of pesticide residues on plants with this innovative technique.

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RESEARCH Open Access

Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin

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Abstract

Background: Pyrethroid insecticides, carbamate and organophosphate are the classes of insecticides commonly used in agriculture for crop protection in Benin. Pyrethroids remain the only class of insecticides recommended by the WHO for impregnation of bed nets. Unfortunately, the high level of pyrethroid resistance in *Anopheles gambiae* s.l., threatens to undermine the success of pyrethroid treated nets. This study focuses on the investigation of agricultural practices in cotton growing areas, and their direct impact on larval populations of *An. gambiae* in surrounding breeding sites.

Methods: The protocol was based on the collection of agro-sociological data where farmers were subjected to semi-structured questionnaires based on the strategies used for crop protection. This was complemented by bioassay tests to assess the susceptibility of malaria vectors to various insecticides. Molecular analysis was performed to characterize the resistance genes and the molecular forms of *An. gambiae*. Insecticide residues in soil samples from breeding sites were investigated to determine major factors that can inhibit the normal growth of mosquito larvae by exposing susceptible and resistant laboratory strains.

Results: There is a common use by local farmers of mineral fertilizer NPK at 200 kg/ha and urea at 50 kg/hectare following insecticide treatments in both the Calendar Control Program (CCP) and the Targeted Intermittent Control Program (TICP). By contrast, no chemicals are involved in Biological Program (BP) where farmers use organic and natural fertilizers which include animal excreta.
Susceptibility test results confirmed a high resistance to DDT. Mean mortality of *An. gambiae* collected from the farms practicing CCP, TICP and BP methods were 33%, 42% and 65% respectively. *An. gambiae* populations from areas using the CCP and TICP programs showed resistance to permethrin with mortality of 50% and 58% respectively. By contrast, bioassay test results of *An. gambiae* from BP areas gave a high level of susceptibility to permethrin with an average mortality of 94%.
Molecular analysis identified *An. gambiae* s.s, and *An. arabiensis* with a high predominance of *An. gambiae s.s* (90%). The two molecular forms, M and S, were also determined with a high frequency of the S form (96%).
The *Kdr* gene seemed the main target- site resistance mechanism detected in CCP, TICP, and BP areas at the rates ranging from 32 to 78%. The frequency of *ace-1R* gene was very low (< 0.1).
The presence of inhibiting factors in soil samples under insecticide treatments were found and affected negatively in delaying the development of *An. gambiae* larval populations.

Conclusions: This research shows that *Kdr* has spread widely in *An. gambiae*, mainly in CCP and TICP areas where pyrethroids are extensively used. To reduce the negative impact of pesticides use in cotton crop protection, the application of BP-like programs, which do not appear to select for vector resistance would be useful. These results

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could serve as scientific evidence of the spread of resistance due to a massive agricultural use of insecticides and contribute to the management of pesticides usage on cotton crops hence reducing the selection pressure of insecticides on *An. gambiae* populations.

Background

Malaria remains a major public health problem in Africa. It is reported to be the most significant cause of morbidity and mortality, resulting in a critical loss of working days [1].

More than 2 billion people in the world are at risk of contracting malaria and one million deaths are recorded yearly of which 90% occur in Sub-Saharan Africa [1]. In Benin, malaria is still the most important disease leading to 67% consultations in local health centres [2]. The strategy of the National Malaria Control Programme (NMCP) is based on effective case management and vector control with Insecticide-Treated Nets (ITN) and Indoor Residual Spraying (IRS).The development of new insecticides for public health use is limited and requires enormous capital and time, making industry reluctant to embark on such ventures. Novel compounds or alternatives are to be sought in the agricultural pesticide pipeline. Several reports have recently shown evidence that the main African malaria vector, *Anopheles gambiae* s.l., has developed a high level of resistance to pyrethroid insecticides as well as to other classes of public health insecticides. While resistance is now spreading throughout Sub-Saharan Africa, reports from Benin and the West African region indicated the highest recorded frequencies of the resistance genes [3-6].

The development of pyrethroid resistance in the primary malaria vectors, *An. gambiae* s.l. and *An. funestus* [7] is a serious concern. In the last decade, the emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in many African countries including Kenya [8], Côte d'Ivoire [9], Benin [10-14], Niger [15], Burkina Faso [16,17], Mali [18], Nigeria [19], South Africa [20], and Cameroun [21]. In the 1960s, the role of selective treatment with organochlorines (OC) in agriculture on resistance of *An. gambiae* was observed in Mali [22]. Evidence of an association between agricultural use of insecticides and the emergence of resistance in malaria vectors has been repeatedly reported. In Côte d'Ivoire and Burkina Faso, N'Guessan *et al.* [23] reported that the level of vector resistance to pyrethroid insecticides increased during the cotton growing season. Higher frequencies of *kdr* alleles were observed in the more intensely farmed cotton production areas of Côte d'Ivoire [9]. In Bukina-Faso, a survey of *kdr* alleles in *An. gambiae* field populations showed also a higher frequency of *kdr* alleles in older cotton areas with a decreasing gradient to non treated areas [17].

Cotton crop protection represents 90% of the insecticide use in West Africa. The control strategies implemented against cotton pest especially *Helicoverpa armigera* required a regular repeated applications of insecticides during the cotton plant growing cycle. As recommended by the Institut National des Recherches Agronomiques du Benin (INRAB), six consecutive treatments are applied at two weeks interval to protect the crop against bollworms, leafworms and sucking pests. These insecticides are essentially composed of pyrethroids (PYs), organophosphates (OPs) which are also the main classes used in public health and a cyclodiene. The majority of cotton farms observed in northern Benin are located in the upland landscape while the lowland covers the major mosquito breeding sites. Thus run-off has been assumed to be the mechanism by which insecticides from agricultural sites reach malaria vector breeding sites, where they exert a huge selection pressure on larval stages of *An. gambiae* s.l. The main malaria vector *An. gambiae* breeds in puddles, stagnant pools and various sites around or within the lowland. During the rainy season, insecticide residues are washed downwards into mosquito breeding sites thus affecting larval population [24]. According to Akogbeto *et al.* [25] in Benin, insecticide treatments against cotton pests are applied twice a month, for a timeframe of three consecutive months (between July and October) each year. These treatment periods coincide with the rainy season and correspond to the period of high mosquito densities. The evidence supports the hypothesis that breeding sites contamination is the result of the coincidence of agricultural pesticide application and seasonal rainfall/runoff.

Alternatively, integrated pest and vector management (IPVM) strategies based on the rational use of chemical protection, has undoubtedly reduced the negative impact of pesticides on humans, and their environment, including the breeding sites of malaria vectors.

This study aimed to assess the impact of control strategies used against cotton pests (relative amount of insecticide) on the frequency and spread of insecticide resistance in *An. gambiae* populations. The study, conducted in northern Benin, compared the BP cotton cultivation sites (absence of pesticides use) with the CCP and TICP cotton cultivation sites where insecticides are extensively used. The study focused on the investigation of agricultural practices using pesticides for the control of cotton pests and their impact on the insecticide

susceptibility of *An. gambiae* populations from surrounding breeding sites.

Methods

Cotton pests control strategies

Three pest management and control strategies are officially recommended in Benin:

(1) The Calendar Control Program (CCP) is based on the conventional treatment which systematically uses the full dosage of insecticides.

(2) The Targeted Intermittent Control Program (TICP) is based on two steps of crop protection [26,27]. The first steps is a protection which follows a conventional pesticide application schedule (every 14 days from the appearance of floral organs), but only the half dose of insecticide are usually applied. The second step includes a modification of the first treatment meaning that the half-dose left over during the earlier observation made the day before treatment would suggest that the pest populations exceed the economic thresholds of damage. The program was established five years ago.

(3) In the biological control program (BP), no chemical is used for plant protection. That program started over the past five years.

The area of the farms applying CCP and TICP was about 4 hectares and usually farmers ploughed sometime individually or work in groups of farmers' organizations. However, in BP sites of 1 hectare, farmers worked under the supervision of technicians from the Beninese Organization for Organic Farming Promotion (OBEPAP) who assisted in the implementation and the survey of good agricultural practices on organic cotton. These areas were characterised by a continual production of cotton crop.

Study sites

The study was conducted in the cotton areas around 8 cities in Benin (Figure 1). The choice of these areas took into account the various strategies of pest control. Semi permanent breeding sites were found in cotton fields where farmers used:

• the CCP around Parakou (2°62 E, 9°33 N), Kandi (2°95 E, 11°16 N) and Banikoara (2°59 E, 11°31 N). This pest management program started thirty years ago and was the main strategy against pest control used by more than 95% of the cotton farmers.

• the TICP around N'dali (2°70 E, 9°84 N), Kandi (3°08 E, 11°27 N) and Banikoara (2°41 E, 11°31 N) started five years ago and applied by 4% of cotton farmers.

• the BP around Kandi (2°92 E, 11°09 N) and Banikoara (2°52 E, 11°29 N) started five years ago and practiced by 1% of cotton farmers.

The annual mean rainfall recorded was about 1,300 mm yearly and characterized by a Sudanian climate with one rainy season (middle of May to October) and one dry season (November-May).

KAP Study on the use of insecticides in cotton farms

To generate adequate information on the use of insecticide on cotton fields, Knowledge Attitude-Practice (KAP) surveys were organized in the study sites. In each site, leaders of farmer's organizations were interviewed using semi-structured questionnaires that focused on the treatment strategies, and the use of insecticides in the farms. Further, qualitative data was collected through direct observations, in-depth interviews and focus group discussions.

Mosquito collections

Mosquito larvae were collected during the rainy season as well as before and during the period of insecticide treatments. The treatment periods started from July to October. During pest control, insecticide residues contaminate mosquito breeding sites whereby they diffuse into the water applying a selection pressure on mosquito larvae. Larvae were collected in the breeding sites of each site and transported to the laboratory of the Centre de Recherche Entomologique de Cotonou, Benin (CREC) for resistance testing. The adults were tested after emergence. A laboratory susceptible strain of *An. gambiae* Kisumu was used as a reference strain to compare the susceptibility levels of the field populations.

Insecticide susceptibility tests

Mosquitoes collected were assayed using WHO discriminating dosages of four insecticides: permethrin (0.75%), DDT (4%), deltamethrin (0.05%) and bendiocarb (0.1%). Four batches of 25 unfed females, aged 2-5 days, were exposed to the diagnostic doses of insecticide treated papers for 1 hour. The twenty five females of *An. gambiae* were introduced into each tube and monitored at different time intervals (10, 15, 20, 30, 45, 60 minutes) the number "knocked-down" recorded. After one hour exposure, mosquitoes were transferred into holding tubes and provided with cotton wools wet with a 10% honey solution. Mortalities were recorded after 24 hours and the susceptibility status of the population was graded according to the WHO protocol [28]. Dead and surviving mosquitoes from this bioassay were separately kept in Carnoy solution at -20°C for further molecular analysis.

Molecular characterization

All *An. gambiae* s.l. were identified to species using PCR [29] and as M and S forms by PCR-RFLP [30]. To detect the presence of *Kdr* mutation in the samples collected from each study site, polymerase chain reaction diagnostic test for detection of *kdr* "Leu-phe" genes was carried out on *An. gambiae* mosquitoes from each study site as

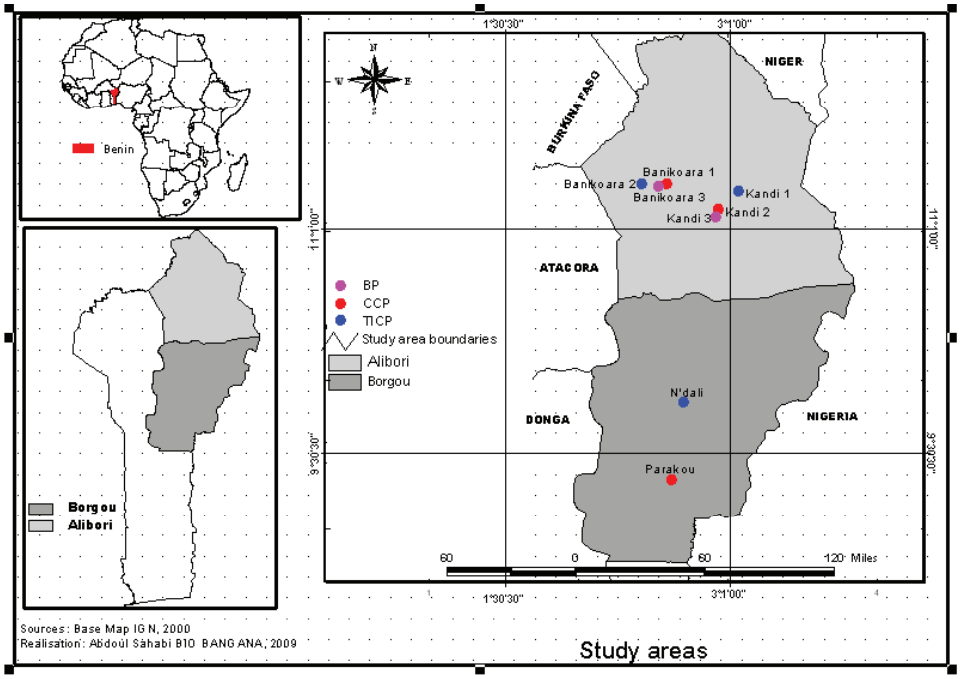

Figure 1 Map of Benin showing the study sites.

described by Martinez-Torres et *al*. [31].The PCR-RFLP diagnostic test was used to detect the presence of G119S gene (*Ace.1* gene) as described by Weill et *al*. [32].

Screening of pesticide residues in soil from agricultural settings

This investigation was performed using indirect bioassays focused on factors which can affect the normal growth of mosquito larvae in cotton breeding sites. The hatching rates of *An. gambiae* eggs and the larval survival during rearing period were assessed in artificial breeding sites made of soil samples collected from different cotton areas. The pyrethroid-susceptible Kisumu strain and the resistant VKPER strain were used to test the presence of insecticide residues in the soil samples by means of mortality rates after exposure. The testing method was based on an artificial breeding site made of a mixture of soil from cotton sites (BP, TICP and CCP programs) and CREC soil used as a control. 100 g of each soil was weight and mixed in 1,000 ml of water. 200 eggs of the susceptible *Kisumu* strain were placed in each artificial breeding site and compared with 200 eggs of VKPER.

Larvae in all artificial breeding sites were fed with similar quantity and type of food (well ground cat biscuits mixed with yeast powder). Daily observation was done in order to record the number and the instars of larvae. This experiment was replicated three times per month.

Data interpretation

The resistant status of mosquito samples was determined according to the WHO criteria [33]:

- Mortality rates is > 97%: the population was considered fully susceptible
- Mortality rates ranged between 80 > × < 97%: resistance suspected in the population
- Mortality rates < 80%, the population was considered resistant to the tested insecticides.

The knockdown times for 50% and 95% of tested mosquitoes (*KdT50* and *KdT95*) were estimated using a log-time probit model [34].

The resistance allele frequency at the *kdr* and *Ace*-1 locus was calculated using Genepop software (version 3.3) as described by Raymond and Rousset [35].

A Fisher's exact test was performed to compare the resistance allele frequency at the *kdr* and *Ace*-1 among the mosquitoes from the different strategies.

An analysis of variance (ANOVA) was performed to compare the percentage of hatching eggs in the different treatments in order to know the impact of insecticide on the normal growth of mosquito larvae in cotton breeding sites.

Results

Knowledge-Attitude -Practice (KAP) investigations

Results from our KAP investigations from June to September 2008 in the cotton growing areas showed a common use by farmers of mineral fertilizer NPK at about 200 kg.ha-1 and urea at about 50 kg.ha-1 in both the CCP and the TICP sites. By contrast, in BP areas, all farmers in this group used organic and natural fertilizers which included animal excreta. In the CCP sites, about 6 pesticide treatments were applied by farmers 45 days after seeding and at two week intervals from flowering. Endosulfan or Tihan® (mixture of spirotetramat + flubendiamide), were sprayed in the first two treatments followed by the mixtures of cyfluthrin + chlorpyrifos ethyl for the 3rd and the 4th treatment and then cypermethrin + dimethoate applied for the last two treatments. In TICP sites, the same treatments at intervals of two weeks were made either at half dose or at a complete full dosage when the threshold of infestation was reached (5 of *H. armigera* larvae observed on 50 plants). In areas practicing Biological Program, farmers apply a mixture of neem or papaya leaves with added chilli and local soap three times before the harvest.

Resistance to insecticides

A total of 1,313 females of *An. gambiae* collected from different sites around Parakou, N'dali, Kandi, and Banikoara were exposed to papers impregnated with discriminating doses of permethrin (0.75%), deltamethrin (0.05%), DDT (4%) and bendiocarb (0.1%).

The knockdown times (*KdT50*, *KdT95*) of *An. gambiae* populations from CCP and TICP sites were significantly longer than that of the susceptible strain Kisumu ($p < 0.05$). However, the *KdT50* for *An. gambiae* from BP site around Kandi was not significantly different from Kisumu (Table 1). Data recorded before and during the period of treatments showed a higher resistance to DDT and permethrin in populations from the CCP and TICP sites compared with those from the BP sites (Figure 2 and Figure 3). All populations of *An. gambiae* mosquitoes were resistant to DDT with an average of 33%, 42% and 65% of mortality respectively for CCP, TICP and BP sites. The mortality difference associated with the different pesticide application strategies was highly significant between BP and CCP programs ($P < 0.05$) but not significant between BP and TICP program (P = 0.56). However, *An. gambiae* populations from BP, CCP and TICP sites were fully susceptible to deltamethrin and bendiocarb (100% of mortality). Permethrin resistance was found in *An. gambiae* populations from CCP and TICP sites with an average mortality of 50% and 58% respectively. However, *An. gambiae* collected from BP sites were more susceptible to permethrin with 94% mortality.

Species identification

A total of 850 *An. gambiae* adults were analysed for species and molecular forms. Most of the mosquitoes collected from all the sites were *An. gambiae* s.s. (90%), which was found in sympatric with a low proportion of *An. arabiensis* (0 to 5%) except in Parakou and N'dali where they were more present (22 to 30% respectively) (Table 2). In *An. gambiae s.s*, the M and S forms were always found in sympatric but the S form was mostly predominant (96%).

Resistance mutations

The *kdr* genotype was scored for 1,400 individuals (100 mosquitoes consistently failed to amplify). The *kdr* gene occurred in S forms (Table 2). The highest frequency of *Kdr* mutation was recorded for the populations from three CCP sites (67-78%) and the lowest (35 and 32%) were found in the populations from BP sites around Kandi and Banikora respectively.

The *Ace-1R* gene was found at very low frequency ranging (from 0.00 to 0.06) in heterozygote *An. gambiae* s.s from the three CCP sites (Table 2). Among *An. gambiae* s.s, there was no mosquito of the M molecular form carrying the *ace-1R* gene.

The resistance allele frequency at the *kdr* was significantly higher in areas where farmers used insecticide for pest control (CCP and TICP) than in those no insecticide is not request (BP) ($p < 0.05$.). However, there is no difference between the resistance allele frequency at the *kdr* from mosquitoes in CCP and TICP strategies ($p > 0.05$).

Pesticide residues

Results of soil samples for pesticide residues analysis showed that artificial breeding sites made with soil from CREC (control) and soil from Biological program (BP) sites were similar with no effect on hatching of *An. gambiae* Kisumu and VKPER strains (Figure 4). Tests with the susceptible *An. gambiae* Kisumu strain gave percentages of hatching equivalent to 80% in control soil (no contact with pesticides) and 75% with soil from BP sites. However, with the pyrethroid resistant strain VKPER the percentages of hatching were 83% and 77% with the control soil and soil from BP sites respectively. The hatching percentages of both strains decreased significantly when

Table 1 Knockdown times (KdT_{50} and KdT_{95}) and mortality of *Anopheles gambiae* s.l. populations from 3 cotton sites after exposure to DDT 4% and permethrin 0.75% and their resistance status

Sites/Strains	Program	Insecticides	N	kdT50 [CI95] (min)	kdT95 [CI95] (min)	% Mortality [Conf lim 95]	Resistance status
		DDT	40	65.1 [57.5-73.4]	152.1 [118.4-228.1]	38 [29.31-46.7]	R
Parakou	CCP	Permethrin	75	35.3 [32.1-38.4]	112.1 [88.2-151.9]	54 [47.49-60.5]	R
		DDT	60	38.1 [29.4-36.5]	65.1 [57.5-86.5]	45 [37.73-52.3]	R
N'dali	TICP	Permethrin	60	19.3 [15.6-22.4]	67.1 [53.2-87.4]	60 [52.8-67.16]	R
		DDT	70	63.1 [60.2-72.3]	135.1 [5.2-184.5]	32 [25.7-38.31]	R
Kandi1	CCP	Permethrin	80	19.3 [15.6-22.4]	87.1 [63.5-138.4]	50 [43.67-56.32]	R
		DDT	50	35.1 [27.1-35.2]	62.5 [54.2-79.1]	43 [35.07-50.92]	R
Kandi 2	TICP	Permethrin	88	15.1 [13.6-20.1]	56.5 [43.2-77.2]	58 [52.00-64]	R
		DDT	60	30.3 [25.4-38.9]	72.6 [50.2-90.5]	66 [59.08-72.92]	R
Kandi 3	BP	Permethrin	80	11.0 [8.7 - 17.6]	23.5 [18.4-35.8]	94 [91.01-96.98]	S
		DDT	65	56.5 [51.7-63.2]	186.9 [146.0-265.2]	35 [28.3041.69]	R
Banikoara 1	CCP	Permethrin	90	24.6 [20.3-29.0]	105.2 [92-130.8]	51[45.04-56.96]	R
		DDT	70	32.4 [25.8-30.2]	60.1 [50.1-78.6]	41 [34.34-47.65]	R
Banikoara 2	TICP	Permethrin	70	18.5 [16.1-22.4]	58.1 [47.4-78.4]	59 [52.35-65.65]	R
		DDT	75	29.1 [24.4-37.6]	70.1 [48.2-88.2]	64 [57.73-70.27]	R
Banikoara 3	BP	Permethrin	80	13.2 [9.4 - 18.2]	25.5 [20.2-37.1]	95 [92.24-97.75]	S
		DDT	100	25.7 [24.3-27.0]	40.7 [38.0-44.5]	98 [96.41-99.58]	S
An. gambiae s.l	*	Permethrin	100	10.9 [9.7-12.0]	18.1[16.0-21.6]	99 [97.87100.13]	S

KdT_{50} , knockdown time in min for 50% mosquitoes; *Kd*T95 , knockdown time in min for 95% mosquitoes; CI, confidence interval at 95%;
*, No program = Control; Conf Lim 95% = confidence interval at 95%.

soil samples from CCP and TICP sites were used. With the TICP soil, *VKPER* hatching was 45% against 25% for the Kisumu strain, whiles the CCP soil gave 34% hatching for VKPER and 11% for Kisumu. In both cases the results showed that the hatching rates were significantly higher ($P < 0.05$) with VKPER than Kisumu when the soil samples tested were from TICP and CCP sites.

Similar results were obtained with the emergence of adults of *VKPER* and Kisumu strains from eggs placed in artificial breeding sites consisting of water and soil samples from CCP and TICP relative to the control (Figure 4). There was no significant difference between the emergence of adults of *VKPER* and *Kisumu* strains breeding in artificial sites made with the soil samples from BP compared with the control (Figure 5).

However, a significant difference ($P < 0.05$) was observed between the emergence of *VKPER* on artificial sites made of soils from TICP (43%) and CCP (35%) sites and Kisumu which gave 20% emergence on TICP and 13% on CCP.

Discussion

The information generated during interviews with cotton farmers and the observations made in cotton fields has confirmed a common use of fertilizers and insecticides in cotton fields. Cotton cultivation requires intensive use of pesticides including insecticides belonging to the two main classes recommended for vector control in public health: organophosphates and pyrethroids. In West Africa, pyrethroid-treated bed nets remain one of the effective tools for malaria vector control and it provides personal protection to individuals who sleep under them. When used by the whole community, bed nets protect collectively against infective mosquito bites by a mass killing effect of the vectors [36].

In Benin, pyrethroids have been extensively introduced in agriculture since 1980s [25]. This factor is probably one of the causes of the selection of strong resistance in *An. gambiae* to permethrin and DDT, particularly in cotton growing areas. Based on recent results, several authors [8-12; 37] have reported that past and current agricultural use of DDT then pyrethroids for crop protection have led to the selection of resistant mosquitoes through insecticide residues accumulated in breeding sites around cotton growing areas. This hypothesis was recently confirmed by Akogbeto *et al.* [25] showing indirectly the presence of pesticide residues in soil and water from vegetable farms and other agricultural activities in Benin that delay or reduce the emergence rates of mosquito larvae.

The use of insecticides in households for public health purposes and massive quantities of pesticides in agricultural settings has been highlighted as a key factor contributing to the emergence of vector resistance. A recent report by Yadouleton et *al*.[37] showed that agricultural practices in urban areas seem to have contributed to the emergence of insecticide resistance in *Anopheles* populations. Our study in vegetable farming systems in Benin

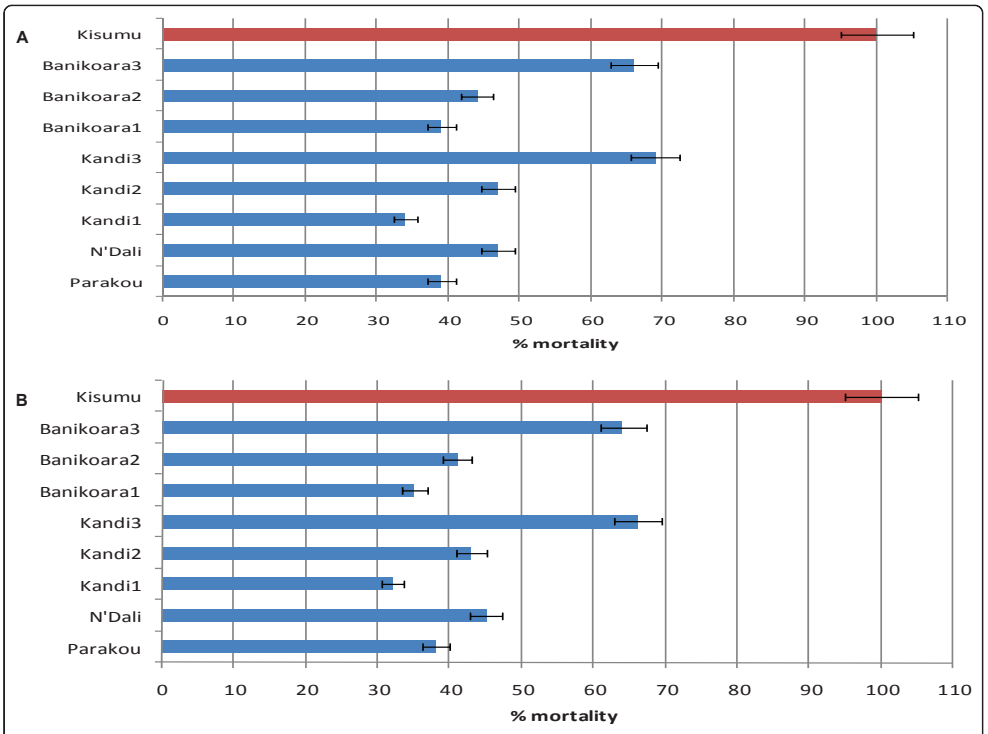

Figure 2 Mortality rates and resistance status of *Anopheles gambiae s.l.* collected before (A) and after (B) cotton treatments to DDT 4% using WHO bioassay tests.

demonstrated that improper use of insecticides to control vegetable pests in urban areas directly exerted a huge selection pressure on mosquito larval populations. The high mortality observed with mosquitoes reared on soils from CCP, TICP sites can be explained by the presence of DDT residues in the soil from those sites and the extensive use of pyrethroids by farmers [38]. Our results showed that the high level of the *kdr-west (*Leu-Phe) gene seemed to be the main resistance mechanism and responsible for the decrease of mortality rates to DDT and permethrin and is more of an ongoing process in *An. gambiae* populations from CCP and TICP sites. The *kdr* gene in the main malaria vector *An. gambiae* was found at high frequency in samples from the sites using insecticide (CCP and TICP) than those with no use of insecticide (BP program). The low frequency of *Kdr* gene in BP localities compared with those from CCP and TICP could be due to the fact that in the past these farmers in BP sites used insecticide to control cotton pests. According to reports by Akogbeto et *al* [25], Djogbénou et *al* [14], and Yadouleton et *al.* [38], the presence of *Kdr* genes in mosquito can be due to external factors that affect mosquitoes as larvae or adults. In 2000, a study in Burkina Faso by Diabate *et al.* [17] reported higher levels of *kdr* alleles frequency in *An. gambiae* collected from cotton-growing areas constantly subjected to insecticide treatments, as compared to the low frequency of *kdr* recorded in rural areas where farmers are restricted to low or no use of pesticides. Despite the use of insecticide in both CCP and TICP sites, the difference in adult mortality rates between CCP and TICP program can be explained by the fact that CCP program uses more insecticide than TICP program.

This study provides clear evidence of the association between the use of insecticides in agriculture and the widespread emergence of insecticide resistance in *Anopheles* species.

Indeed, in Benin, insecticide treatments against pests in cotton plantations are carried out twice a month, for

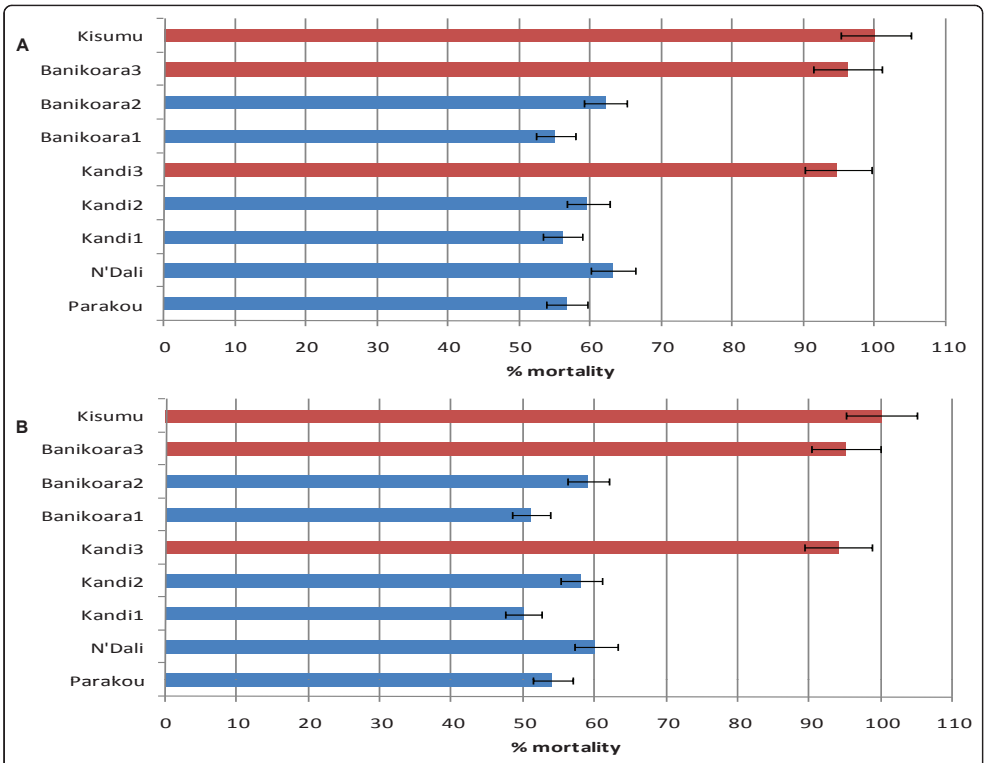

Figure 3 Mortality rates and resistance status of *Anopheles gambiae s.l.* collected before(A) and after (B) cotton treatments to permethrin 0.75% using WHO bioassay tests.

an average period time of three months (between July and October) per year. That treatment period during the rainy season correspond with the period of high mosquito densities because *Anopheles* populations have numerous breeding sites particularly in cultivated areas.

As reported by Akogbeto *et al* [26], some populations of *An. gambiae* may lay their eggs in breeding sites containing insecticide residues. These eggs undergo a selection pressure from agricultural pesticides, which leads to the emergence of resistant strains. There is clear

Table 2 Species and molecular forms identification within *Anopheles gambiae* complex and the frequency of *Kdr* and *Ace-1R* mutations in *Anopheles gambiae* s.s. in Benin

	Species[a]		Mol. Form		*Kdr* mutation				*Ace.1* mutation			
Locality	%Aa	%Ag	%M	%S	SS	RS	RR	F(R)	SS	RS	RR	F(R)
Parakou[1](92)	22	78	5	95	12	35	45	0.68	30	02	0	0.06
N'dali[2](92)	30	70	3	97	22	40	30	0.54	25	0	0	0.00
Kandi[1](92)	5	95	0	100	7	45	40	0.67	30	02	0	0,06
Kandi[2](92)	4	96	0	100	28	34	30	0.51	30	01	0	0.03
Kandi[3](90)	0	100	0	100	45	25	20	0.35	30	0	0	0.00
Banikoara[1](102)	3	97	0	100	12	36	54	0.78	30	02	0	0.06
Banikoara[2](92)	02	98	0	100	14	48	30	0.59	30	0	0	0.00
Banikoara[3](96)	02	98	2	98	48	36	12	0.32	25	0	0	0.00

(a) Aa = *An. arabiensis*; Ag = *An. gambiae s.s.*, superscripts in column; 1 = Control strategies: 1 = CCP; 2 = TICP; 3 = BP.

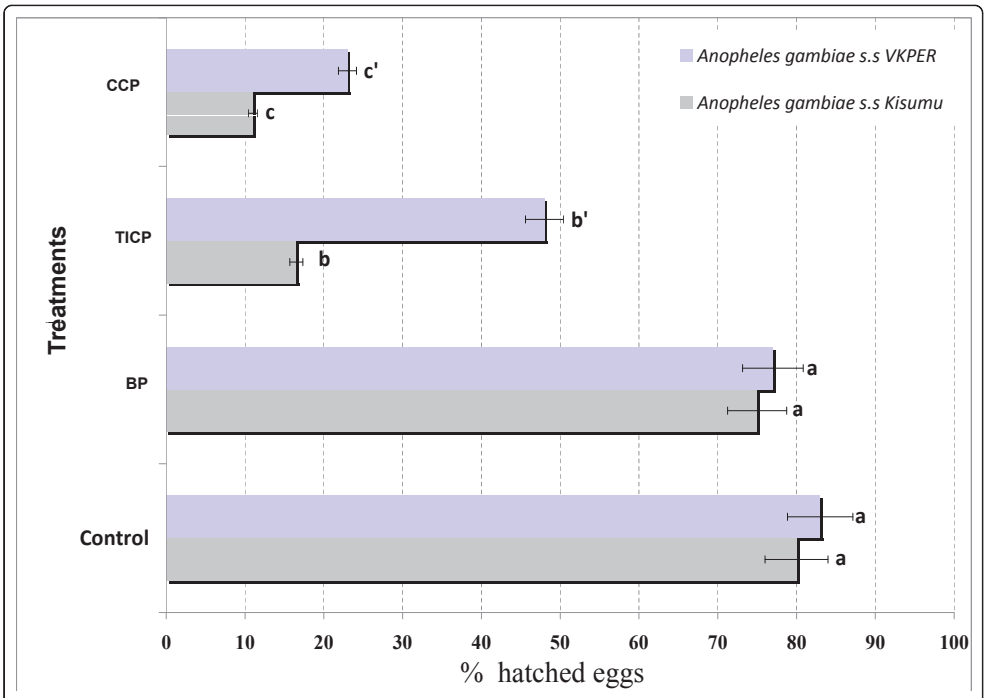

Figure 4 Percentage of eggs hatching of *An. gambiae* in water collected from breeding sites inside CCP program (in Parakou), TICP program (in N'Dali), BP program (in Banikoara) compared with distilled water (as control).

evidence on the implication of agricultural use of insecticides in the selection of resistance in the major malaria vectors. Our results agree with the work of Akogbeto *et al* [25] and confirm once again the impact of the extensive use of insecticides in cotton crop protection on the emergence of insecticide resistance in *An. gambiae* populations. Moreover, in CCP and TICP program, some farmers used insecticides belonging to the organophosphate classes. *Ace-1R* gene is the main resistance mechanism of *An. gambiae* s.l. to organophosphates and carbamates also. The present study, has shown that the *ace-1R* gene is present at low frequency (ranging from 0.01 to 0.09), but only in CCP and TICP program. However, previous field surveys on *An. gambiae s.l.* populations of South-Western

Burkina Faso by Djogbenou *et al* [14] in cotton fields showed that *ace-1R* gene was the main resistance mechanism in *An. gambiae* s.l.

However the National Malaria Control Program (NMCP) in Benin has started scaling up Long Lasting Insecticidal Nets (LLINs) and carbamate for Indoor Residual Spraying (IRS) countrywide for malaria control. The challenge to find effective strategies to manage insecticide resistance in *Anopheles gambiae* remains a high priority and an urgent need particularly in Benin where pyrethroid resistance has been reported with a clear evidence in experimental huts of reduced efficacy of ITNs and IRS [39]. One of the strategies will be to remove pyrethroids from agricultural pest control and leave these classes of insecticides for public health purposes and promote other classes of insecticides such as Spinosad which does not show cross resistance to pyrethroids (i.e. the *kdr* gene).

Conclusions

With the spread of *Kdr* allele frequency from CCP and TICP programmes, to reduce the emergence of insecticide resistance in *An. gambiae* population, African governments would be better advised to promote the BP cotton or genetically modified cotton such as *Bt* Cotton (*Bacillus thurengiensis*) which require lower pesticide than the cotton with CCP and TICP programme and

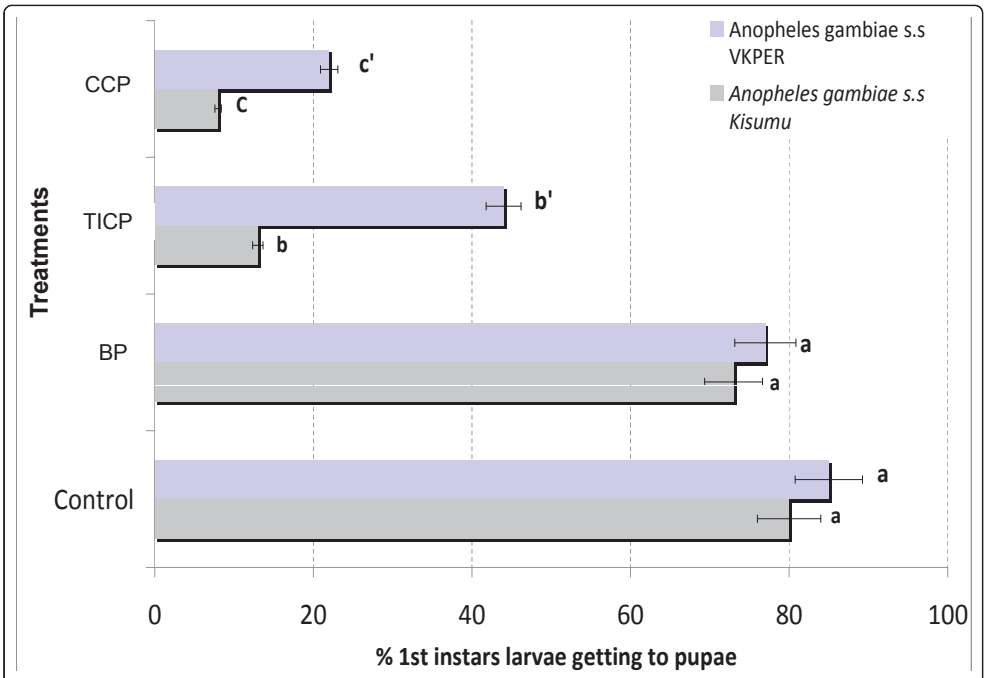

Figure 5 Percentage of emerging adults of *An gambiae* reared in water collected from breeding sites inside CCP program (in Parakou), TICP program (in N'Dali), BP program (in Banikoara) compared with distilled water (as control). - **A**: before treatments - **B**: after treatments • Areas for CCP programs: Parakou; Kandi 1 and Banikoara1 • Areas for TICP programs: N'dali, Kandi 2 and Banikoara 2 • Areas for BP programs: Kandi 3 and Banikoara 3

would permit to suppress the massive use of pyrethroid insecticides.

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Authors' contributions
AY carried out field experiments, collected, analysed, interpreted data and wrote the manuscript. TM, GP reviewed the manuscript and contributed to the design of the study and substantially helped in drafting the manuscript, and revised the manuscript. FC**,** LD, RD, IG and MB contributed to the design of the study. AA helped in drafting and reviewing the manuscript and RA helped with the activities. TM, MA conceived and designed the study, supervised fields and laboratory procedures, and reads the manuscripts. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Characterization of Health and Environmental Risks of Pesticide Use in Market-Gardening in the Rural City of Tori-Bossito in Benin, West Africa

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ABSTRACT

A study on the use of pesticides in market-gardening production was carried out on 108 market-gardeners in the rural city of Tori-Bossito in Southern Benin. The objective of the study was to characterize the potential risks of pesticides usage by farmers and the impacts on their health and on the environment. Two risk indexes were calculated for each pesticide: an environmental risk index (ERI) and a health risk index (HRI). First stage larva of the mosquito *Aedes aegypti* were used as bio-indicator for detecting insecticide residue in vegetable before their harvesting on the farms. The highest ERI were obtained for carbofuran, chlorpyriphos ethyl and endosulfan. Pesticide residues were found in 42% of the samples of leaves of eggplant, cucumber, amaranth and solanum. Vegetables growers used pesticides that may be highly hazardous and which were not registered in most cases. These situations could have unexpected consequences including the exposure of consumers to health hazards.

Keywords: Vegetable; Pesticide Residues; Bio-Indicator; Risk Index; Environment; Health

1. Introduction

Pesticides are increasingly used by farmers in Sub-Saharan Africa to protect their crops. Pesticides are used to destroy, neutralize insects, vermin, all kinds of human disease carriers, and other harmful or awkward plants or animal species. They help during the production, transformation, storage, transportation or distribution of foodstuffs, agricultural products, wood, and derived products. [1]. The use of these products requires some rules and procedures in order to avoid endangering human beings and the environment. Human health issues related to pesticide's use in agriculture have been noted in all regions of the world [2,3]. The environmental consequences related to the use of pesticides relate to things such as air quality, soil, water and biodiversity [4]. In West Africa, a pesticide contamination of the ground water was observed in farming area of Niayes in Senegal [5]. A contamination of ground water by the organophosphate and organochlorine pesticides was reported in several areas in Ivory Coast [6]. After the spreading of pesticide on plants, the farmer generally needs to withdraw for some time (depending on the strength of the product) before harvesting in order to prevent the contamination of the crops. We noted however that this requirement is frequently bypassed. For instance, residues amounts exceeding 0.5 µg/g for the organochlorine (DDT, Endrin, Heptachlore) were detected in vegetables in Southern-Benin [7]. Like the majority of Sub-Saharan Africa farmers, those in the rural city of Tori-Bossito know little about the real toxicity of the pesticides and their sedentary use modes [8].

The aim of this study was to characterize by toxicological, ecotoxicological and biological indicators, the potential hazards for human health and the environment of the pesticides used by the small rural farmers of Tori-Bossito.

2. Materials and Methods

The study was carried out in the rural city of Tori-Bossito, located 30 km away from the city of Cotonou, in Southern Benin. It covers 328 km^2 of land and has a huge area of mangroves which are very important for the

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farming and gardening activities in the region. We chose Tori-Bossito for this study because gardening is one of the primary income sources of the populations.

2.1. Survey and Computation of the Risk Indexes

A descriptive and analytical study was carried out to characterize the environmental and health risks related to the use of the agricultural pesticides in the rural city of Tori-Bossito. Hundred and eight gardeners were surveyed to obtain the information relative to pesticide usage in 2008. The information on the bottles and packaging of the pesticides were collected. Based on the information collected using the questionnaires and datas indexed in databases on the pesticides' toxicological and eco-toxicological properties, the health hazards and environmental risk indexes were calculated using the pesticides risk indicator of Quebec-IRPeQ developed by the Quebec National institute of public health (INSPQ), the Quebec ministry for Sustainable development, the Environment and the Parks (MDDEP) and the Quebec Ministry for Agriculture, Fisheries and Food (MAPAQ) [9]. The toxicological data used to compute the indexes was obtained in "The Manual E-Pesticide" (Wise & Loveys Information Ltd Services, 2001) [10], in the European database on the properties of the pesticides "Footprint PPDB" [11] and in the database of "SAgE pesticides" developed by the INSPQ, the MDDEP and the MAPAQ [12]. The choice of the indicator of risks of the pesticides (IRPeQ) was made by considering the availability of the tool, its ease of use depending on data accessibility for the most active ingredients. IRPeQ calculates a human health risk index (HRI) and an environment risk index (ERI). The computation of the HRI considers all the toxicological properties of the active ingredients (acute toxicity, chronic toxicity modulated by a factor related with persistence and bio-accumulation) and certain properties of the commercial products (maximum amount recommended per hectare, and type of formulation). ERI incorporates the ecotoxicological impact of the active ingredients (a.i) on the terrestrial invertebrates, the birds, the aquatic creatures, and certain physio-chemical parameters (bio-accumulation, persistence in the ground and mobility).

$$\mathrm{HRI}_{\text{active ingredient}} = \frac{TRI * LFf * FCP}{10}$$

$$\mathrm{ERI}_{\text{active ingredient}} = \left[1.75 * (T + O) + A + M + P + B + 1\right]^2$$

$$\mathrm{HRI}_{\text{pesticide}} = \sum \mathrm{HRI}_{\text{active ingredient}}$$

$$\mathrm{ERI}_{\text{pesticide}} = \sum \mathrm{ERI}_{\text{active ingredient}}$$

2.2. Residual Amounts of Pesticides

Samples of vegetable sheets and soils were taken to evaluate the presence of pesticide residues using a biological indicator of toxicity [13]. This method does not aim to identify the molecules present in samples, but at comparing the toxic effect of the extracts of sheets or soils on the first larvae stages of *Aedes aegypti* to the effect of a reference molecule, the deltamethrin, a crop protection agent recommended for commercial gardening in Benin. The deltamethrin was used as sentinel molecule because of its strong toxicity (LD_{50} of 0.4 µg/L) on the larvae of *Aedes aegypti* strain S-Be from north of Benin, but also because it belongs to the family of the pyrethrinoids. Indeed these insecticides are frequently used in Benin, either alone or combined with organophosphates, for crop protection [8].

In the market-gardening perimeters of Tori-Bossito, 24 samples of plant were taken in 17 parcels before harvest. Overall, 11 different plants were sampled. To evaluate the evolution over time of the amount of insecticide residue in the cabbage sheets and the surface layer of the ground after a treatment, samples of cabbage sheets and soils were taken one hour before and after treatment with DECIS 12.5EC to 35 g a.i/ha (active ingredient per hectare). Other daily samplings were conducted on the tenth day after treatment. For each sample, 200 mg of vegetable or 60 mg of soil were each put in a tube in which respectively 10 ml and 40 ml of ethanol were added to extract the residues from pesticides in 24 hours. A volume of 0.1 ml of extract was added to the contents of a polystyrene goblet containing 9.9 ml of water with 20 larvae of first stage of *Aedes aegypti* (with three repetitions for each sample). The counting of mortality was made 24 hours after application. The test was taken again with two dilutions 1/10 and 1/100 in the event of total mortality. Natural mortality was taken into account by tests with distilled water containing ethanol 1%. A commercial formulation of Decis 12.5 EC made of deltamethrin with 12.5 g/L was used to establish the calibration line on the larvae of *Aedes aegypti*. A dilution to the 1/200,000 in distilled water was carried out before making a series of ten successive dilutions giving a mortality ranging between 1% and 99% (**Figure 1**). The residual amount of pesticides in the vegetable sheets was evaluated and recorded in microgram per gram of equivalent-deltamethrin starting from the calibration curve.

3. Results

3.1. Human Health and Environmental Risks of the Pesticides

Health Risks Indexes (HRI)

In the results of our analysis, some active ingredients

such as endosulfan, chlorpyriphos-ethyl and maneb presented simultaneously high acute and chronic risks (**Table 1**).

Others like lambdacyhalothrin, acetamiprid, metalaxyl and copper oxyd presented a high acute risk, but a weak chronic risk. Active ingredients such as mancozeb, carbendazim, thiophanate-methyl presented a high chronic risk and a weak acute risk. The maneb and the acetamiprid gave the weakest factor related to persistence in the environment and the bio-accumulation on human. The highest toxicological risk indexes (TRI) and the highest health risk indexes (HRI) were obtained for endosulfan, chlorpyriphos-ethyl and mancozeb (**Table 2**). As far as the commercial preparations are concerned, there are the formulations of endosulfan (COTOFAN 350.EC and THIONEX 350.EC), of chlorpyriphos-ethyl (DURSBAN) and the mancozeb (FOKO) which presented the highest risks for the human health with respectively indexes of 2290, 3499 and 3538 (**Table 3**). Formulations of DECIS 12.EC, TOPSIN M and the COTALM P 218.EC had a slightly lower risk level. The formulation of COTALM P 218.EC was the most used by the producers of Tori-Bossito (84.2% of the vegetable growers). The fungicide CALLOMIL 72WP (metalaxyl and copper oxyd) presented the lowest risk of health impact.

3.2. Potential Presence of Residues of Pesticide in the Samples Analysis

The impact of the deltamethrin on the first stage larvae of *Aedes aegypti* is illustrated by **Figure 1**. The mortality of the larvae was observable starting from 0.1 µg/L of deltamethrin. Mortality was total from 1 µg/L of deltamethrin. The results of the vegetables samples taken on plants before harvest showed the potential presence of residues of pesticides in 41.7% of the collected samples (**Table 4**).

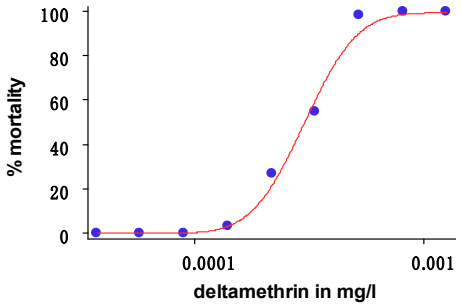

Figure 1. Toxicity of deltamethrin (DECIS 12.5 EC) on *Aedes aegypti* L1 larva (S-Be strain).

Table 1. Values of the parameters entering in calculation of health risk index.

Actives ingredients	Acute risks	Chronic risks	Fper	LFf	FCP	TRI	HRI
Endosulfan	20	29	3	2	1	11449	2290
Lambdacyhalothrin	40	2	3	2	0,5	2116	212
Cyperméthrin	16	25	3	2	0,5	8281	828
Acetamiprid	24	4	1	2	0,5	784	78
Chlorpyriphos-ethyl	22	37	3	2	1	17689	3538
Profenophos	18	18	3	2	1	5184	1037
Deltamethrin	14	37	3	2	1	15625	1562
Cyfluthrin	15	26	2.5	2	0.5	6400	640
Mancozeb	8	50	2	2	1.5	11664	3499
Carbofuran	18	25	2	1	2	4624	925
Carbendazim	4	41	2	2	1	7396	1479
Metalaxyl	21	1	2.5	2	1	552	110
Cooper oxyd	24	3	3	2	1	1089	218
Thiophanate-methyl	7	38	2	2	1	6889	1378
Maneb	20	53	1	2	1.5	5329	1599

Fper: Factor taking into account the persistence and the potential of bio-accumulation in human; LFf: Loading factor related to the type of formulation; FCP: Factor of compensation taking into account the concentration of the active ingredient in the formulation as well as the dose recommended per hectare; TRI: Toxicological Risk Index; HRI: Health Risk Index.

Table 2. Values of the parameters entering in calculation of environment risk index.

Actives ingredients	T	O	A	M	P	B	IRE
Endosulfan	4	2	4	0	2	2	380
Lambdacyhalothrin	0	0	3	0	0	2	36
Cyperméthrin	4	0	4	0	1.5	2	240
Acetamiprid	2	2	0	0	0	0	12
Chlorpyriphos-ethyl	4	3	4	0	1	2	410
Profenophos	4	2	3	0	0	0	210
Deltamethrin	4	0	3	0	0	2	169
Cyfluthrin	4	0	4	0	0.5	2	210
Mancozeb	0	0	4	0	0.5	0	30
Carbofuran	4	4	4	4	1	0	576
Carbendazim	4	0	3	1.5	0	0	156
Metalaxyl	0	0	0	1.5	1	0	12
Cooper oxyd	0	0	2	0	4	0	49
Thiophanate-methyl	2	0	1	0	0	0	30
Maneb	0	0	3	0	0	0	16

T:Impact on the terrestrial invertebrates; O:Impact on birds; A: Impact on the watering organisms; M:Mobility of the active ingredient; P: Persistence in the soil; B: Bio-accumulation; ERI: Environmental Risk Index.

Table 3. Environment and Health risks indexes of pesticides.

Pesticides	Actives ingredients	Farmer's use level (%)	HRI	ERI
DIAFURAN	Carbofuran	2.8	925	576
CYFLUTHRALM P318EC	Cyfluthrin	0.9	1677	420
	Profenofos			
DURSBAN	Chlorpyriphos-ethyl	0.9	3538	410
COTOFAN 350EC	Endosulfan	3.7	2290	380
THIONEX 350EC	Endosulfan	0.9	2290	380
CAPT 88EC	Cypermethrin	1.9	906	252
	Acetamiprid			
COTALM P218EC	Lambdacyhalothrin	84.2	1249	246
	Profenofos			
CYPERCAL 50EC	Cypermethrin	3.7	828	240
CYDIM C50	Cypermethrin	0.9	828	240
CYPER D	Cypermethrin	0.9	828	240
DECIS 15EC	Deltamethrin	4.6	1562	169
BENDAZIM 50WP	Carbendazim	1.9	1479	156
CALLOMILL 72WP	Metalaxyl	1.9	328	61
	Cooper oxyd			
TOPSIN M	Thiophanate-methyl	29.6	1378	30
FOKO	Mancozeb	33.3	3499	30
TRIMANGOL 80	Maneb	0.9	1599	16
IVORY	Maneb	1.9	1599	16

Table 4. Estimation of pesticide residues (in mg of equivalent deltamethrin per kg of leave) in 24 samples of leaves from 11 plants before harvest in 4 market-gardening in Tori-Bossito.

Samples	Market-gardening perimeters of Tori-Bossito			
	Hla	Dohinoko	Ananvié	Houédaga
Cabbage	0.03	-	-	-
Solanum	Nd	-	-	4.75
Eggplant	Nd	-	1.17	0.04
Cucumber	0.07 - 0.11	-	-	-
Bean	Nd	-	-	-
Zucchnini	Nd	-	-	-
Celosia	-	0.09	-	-
Amaranth	-	0.04 - 0.07	-	-
Vernonique	-	Nd	-	-
Squeaky fiddle	-	Nd	-	-
Tomato	-	-	0.04	-

*Nd: Not detected, residual insecticide content expressed mg of deltaméthrine/kg equivalent of leaves.

The most important residual amounts were found in sheets of eggplant and solanum (respectively 1.17 and 4.75 ppm of equivalent-deltamethrin). In the other positive samples coming from both traditional vegetables (amaranthe, celosy, solanum) and "exotic" vegetables (cabbage, tomato, eggplant, cucumber), the amounts were lower than 0.1 ppm of equivalent-deltamethrin.

One hour before the spreading of the DECIS 12.5 EC, no residue of pesticide was detected in the samples of sheets of cabbages and soils (**Figure 2**). One hour after treatment, the residual amount of deltamethrin detected in the cabbage sheets was about 500 ppb (ng/g of sheets) and of 90 ppb (ng/g of soils) in the soils. This residual amount of deltamethrin decreased regularly with time to reach 8 ppb at the end of 10 days. In the samples of soils, the amount of deltamethrin remained stable until the second day after treatment. Beyond two days after treatment, no insecticide residue was detected in the soils.

4. Discussions

4.1. Evaluation of the Environmental and Health Risks of the Pesticides

The indicator of risk for the environment showed the carbofuran as being the most dangerous product for the environment among all the listed active ingredients. Moreover, a high quantity of the active ingredient must be used (5 kg ma/ha) for an effective stabilization of soils against nematodes, according to the inscriptions present on packaging. According to the surveyed vegetable growers, Carbofuran is the only nematicide product available in Benin. ERI confirmed that Carbofuran actually belongs to the 1b category (highly dangerous) of the World Health Organization (WHO) pesticides classification [14].

ERI also classified chlorpyriphos-ethyl and Endosulfan as dangerous for the environment after Carbofuran. Endosulfan was recently prohibited in Benin like in most Sub-Saharan African countries, especially the members of the Inter-State Committee for the Fight against Dryness in the Sahel (CILSS) [15]. It is very neurotoxic and was classified in the Ib category (very highly dangerous) by the US EPA [16] and in category II (moderately dangerous) by WHO [14]. Cases of intoxication and deaths were blamed on Endosulfan in Benin [17]. Acetamiprid is classified as the least dangerous for the environment. Nevertheless, its use is risky for birds and terrestrial invertebrates such as the bees [10,11].

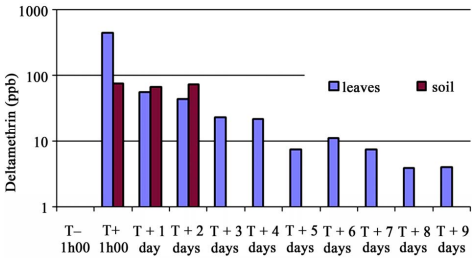

Figure 2. Residual quantity of deltamethrin in cabbage plots and soils after a spray (T) with Decis 12.5 EC at 35 g a.i./ha.

The HRI classified Mancozeb, Chlorpyriphos-ethyl and Endosulfan as very risky for producers and consumers' health. These active ingredients have both a high chronic toxicity risks and a high potential of bio-accumulation on human. Mancozeb was used by one third of the producers as a fungicide. Regular exposure to this active ingredient could induce occupational diseases in the long run. Indeed Mancozeb has a strong environmental persistence and a high potential of bio-accumulation. It is genotoxic and could have an impact on human reproduction [18]. It could be replaced by biological fungicides such as Bacillus subtilis which would equally fight diseases induced by mushrooms [19].

COTALM P218EC (mixture of lambdacyhalothrin and profenofos) presents a health and environmental hazard relatively lower in comparison with formulations of endosulfan and chlorpyriphos-ethyl which have a higher risk of acute toxicity (high eyepiece and cutaneous irritation). It is riskier for birds, terrestrial and aquatic living things. It is used by producers because of its low cost but also for its broad spectrum of activity. Lambdacyhalothrin like any pyrethrinoid has also a toxic and repulsive effect on useful insects (parasitoïdes): this increases the risk of vermin's overpopulation. It would be preferable to replace it with Spinosad (Laser), a bio-insecticide frequently used on tomato in Benin.

IRPeQ enabled us to calculate the environmental and health risk in relation to the amounts applied per hectare. For the calculation of ERI, the ecotoxicological properties were taken into account to estimate the impact of the active ingredients on aquatic creatures, invertebrates and birds. It also considers several physico-chemical parameters such as mobility, persistence in the ground, bio-accumulation and other characteristics related to the commercial preparation. For the calculation of the HRI, the acute and chronic toxicological characteristics, environmental persistence and the potential of bio-accumulation of the products are considered. Next, the types of formulation and equipment come to modulate the health hazard. This indicator allows us to compare various pesticides, and make decisions based on environmental and public health criteria [9]. This indicator can be modified according to whether it is used for provincial assessments or on the farm. In the first case, the risks indexes are calculated on the basis of the reference amount applied per hectare whereas in the case of application to the farm, the module IRPeQ-express let the farmers integrate the actual quantity used in their calculation [20]. This module is distinct but complementary to the "SAgEpesticides" which wants to be especially a tool facilitating the choice of low-risks pesticides. IRPeQ also accounts for the drift and the height of vegetation in the ERI computation. IRPeQ considers that the good practices are respected by the workers so for instance, it won't assess whether or not, growers are wearing protective gears. This variable can easily be integrated into an exercise of risks analysis referring to various scenarios of exposure without being a decision-making tool. The model can however be updated to adapt to techniques used in tropical Africa where some producers ignore the measurements and safety rules, and simply use branches of plants to sprinkle the insecticidal pulp and thus to make possible to quantify the risk for a producer [8]. One limitation of the risk indicators is that the underlying data is not available for all the active ingredients. This was also mentioned by [21]. Due to the lack of clearly defined benchmarks for the risk indicators, the results obtained by score aggregation will lack traceability and make it hard to statute on whether or not a product is safe for use [22]. They however let you compare active ingredients and commercial preparations to choose the least risky for human health and environment. The same active ingredient in two different commercial preparations can have different environmental and health risks indexes because guidelines and reference amounts vary for each commercial preparation. Sometimes, entry variables are highly weighted [22,23]. IRPeQ however doesn't overweight certain variables compared to others. It is a complex indicator which aggregates the scores of several parameters to accurately depict reality.

4.2. Biological Method of Detection of Pesticides Residues

The strongest residual amounts of pesticides were found in samples of eggplant and solanum (respectively 1.17 and 4.75 ppm of equivalent-deltamethrin). To draw a comparison with food poisoning risk, these doses exceed the maximum limit of residue in vegetables accepted in the European Union for deltamethrin (approximately 0.5 ppm) [24]. One explanation may be that these plants were treated with other active ingredients a few days before the samples were taken. The biological indicator used found traces of residual contents (5 to 10 ppb of deltamethrin) up to 10 days after a treatment in spite of the water-spraying carried out twice everyday (morning and evening). These make it a sensitive indicator in addition to being inexpensive. A little less than half of the vegetable samples contained less than 0.5 ppm of deltamethrin equivalent. This represents an intoxication threat. The amounts can be underestimated if the growers use pesticides less toxic than deltamethrin on *Aedes aegypti* larvae. Generally the producers sell these vegetables to wholesalers or retailers who made them directly available to consumers. It's therefore very important to educate buyers about the importance of washing thoroughly the vegetables before consumption. The longer the lifespan of the pesticide, the higher are its risks. The

residues of pesticides are not detected in the samples of soils beyond two days after spreading of the DECIS 12.5EC. This could be explained by the sandy nature of the soil and frequent irrigation. But the absence of residues in the soils doesn't mean that these molecules were destroyed. By infiltration they could have infiltrated the groundwater and thereby jeopardize water quality. This biological pesticides residues detection method is used only as indicator of pollution because it doesn't reveal the active ingredients contained in the samples [13]. It could be coupled to more expensive but more precise methods of analysis such as chromatography coupled with mass spectrometry [7,25,26]. The method only detects residues having a lethal effect on the larvae of *Aedes aegypti*. Considering the fact that pyrethrinoid are used by almost 85% of producers in Tori-Bossito and that the larva of *Aedes aegypti* is very sensitive to these products, we could infer that these plant health products contribute to the toxicity observed in the larvae. It becomes therefore critical from a public health stand-point to educate the vegetable growers on the risks of the plant health products.

5. Conclusion

Our investigation enabled us to identify the formulations of pesticides used in commercial gardening and to target those with a high potential impact on human health and on the environment. The pesticides having the highest risks were also the least frequently used. The biological method of detection indicates the potential presence of residues of pesticides in certain samples of vegetables. Frequent analysis of residues of pesticides with methods of analytical chemistry will enhance the specificity of the observed results. For the safety of the producers, distributors and consumers, it appears necessary that public authorities pay a closer attention to the marketing and use of plant health products and develop alternatives methods like the physical fight [27,28].

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Biotype status and resistance to neonicotinoids and carbosulfan in *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Burkina Faso, West Africa

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Bemisia tabaci Gennadius is a one of the major pests of cotton crops worldwide. In Burkina Faso, data on resistance to neonicotinoids and carbamate insecticides related to species/biotypes remain very scarce. To evaluate the resistance status of *B. tabaci* in Burkina Faso, four insecticides were tested using the leaf dip method on 10 field populations collected from cotton. The status of biotypes was also determined. Two biotypes, Q and ASL, were recorded. Only Q1 group was detected in Q biotype. A significant resistance to neonicotinoids and carbosulfan was shown in most of the populations tested. The highest resistance ratios (RRs) were recorded in populations from locations exhibiting only the Q1. However, the populations comprising a mix of Q1 and ASL appeared to be more susceptible to insecticides. Resistance to neonicotinoids may be related not only to the biotype status but also to the environmental factors and agricultural practices. The exclusive use of neonicotinoids against whiteflies on cotton in Burkina Faso is expected to continue to select for the resistant Q biotype and might threaten the short-term control of whitefly populations, thereby increasing the risk of outbreaks in different host plants and begomovirus transmission.

Keywords: *Bemisia tabaci*; biotype; carbamate; insecticide resistance; neonicotinoid

1. Introduction

The sweet potato whitefly *Bemisia tabaci* (Gennadius) is one of the most serious pests of many agricultural and ornamental crops in all tropical and subtropical regions. *Bemisia tabaci* is considered to be a complex of morphologically indistinguishable species (as opposed to intraspecies races), which vary greatly with respect to host range, fecundity, insecticide resistance, ability to transmit plant viruses, and induction of plant disorders (Brown et al. 1995; Beitia et al. 1997; Perring 2001; Simon et al. 2003; Devine et al. 2004; De Barro et al. 2011). However, the taxonomy and systematics of the pest remain controversial. To date, 28 putative cryptic species within the *B. tabaci* complex have been identified (McKenzie et al. 2009; Dinsdale et al. 2010; Shu-Sheng et al. 2012). Among the 28 putative species, the Middle East–Asia Minor 1 (MEAM1), known commonly as biotypes B and B2, and the Mediterranean (MED), known as biotypes Q, J and L, and Subsaharan Africa Silverleaf biotypes species (ASL), are recognized as predominant in many areas (Dinsdale et al. 2010). In the Q biotype, three groups were recently designated as Q1 (Western Mediterranean populations), Q2 (Middle Eastern populations) (Chu et al. 2008), and Q3, restricted to populations from Burkina Faso (Gueguen et al. 2010). (Hereafter, we have retained the commonly used term "biotype" to link this study with previous literature.)

Life-history traits such as resource exploitation, and resistance to insecticides may affect the distribution and frequency of the different members of the *B. tabaci* species complex (Pascual and Callejas 2004; Horowitz et al. 2005; Crowder et al. 2010). In agricultural areas, human activities, including cultivation practices, the use of cultivated plants and pesticide treatments, create an intense selection pressure on populations and may have a major influence on population demographics and spatial distribution patterns (Reitz and Trumble 2002; Crowder et al. 2010).

In West Africa, population outbreaks were reported in 1998 in cotton fields in Burkina Faso, Mali and Côte-d'Ivoire, inducing severe crop damage (Otoidobiga et al. 2002). The resulting losses severely affected economic activity of these countries, since cotton production and distribution is one of the main financial resources, especially in Burkina Faso. In addition, monitoring of population dynamics on cotton in this country revealed high levels of *B. tabaci* at the boll opening stage (Gnankiné et al. 2007). At this stage, *B. tabaci* causes damage indirectly through

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honeydew excretion, causing the cotton foliage and bolls to become sticky. Consequently, the recommended pest management strategy at this stage is two foliar neonicotinoid treatments against whiteflies in combination with a pyrethroid against bollworms (Gnankiné et al. 2007). Farmers generally spray a mixture of acetamiprid and cypermethrin. This selects for resistance particularly observed in Burkina Faso (Gnankiné et al. 2002; Otoidobiga et al. 2002; Houndété et al. 2010a). Recently, Houndété et al. (2010a) showed resistance of *B. tabaci* to pyrethroids, such as deltamethrin and bifenthrin, to organophosphates, such as dimethoate and chlorpyriphos ethyl, and to neonicotinoids, such as acetamiprid and thiamethoxam. Unfortunately, the biotype in that case was not the same as that collected in Burkina Faso in connection with agricultural practices. The presence of Q1 living in sympatry with the local biotype, Sub-saharan Africa Silverleafing (ASL), has been observed on cotton and vegetable crops (Gueguen et al. 2010; Gnankiné et al. 2012). Q1 was shown to be dominant in Burkina Faso but was not detected in Benin and Togo where ASL was the only biotype (Gnankiné et al. 2012). In these ASL populations, resistance to organophophates and pyrethroids was highlighted (Houndeté et al. 2010a). On the contrary, no neonicotinoid resistance was detected in populations from Benin.

To prevent outbreaks and to achieve sustainable control of this pest, the susceptibility of whitefly to particular pesticides must be assessed with precisely identified populations, human activities and environmental factors. In Spain, the Q biotype exhibited a greater degree of pesticide resistance than did the B biotype, which had a higher fecundity and superior competitive abilities (Pascual and Callejas 2004). In Israel, the Q biotype excluded B biotype when insecticides were used, whereas B biotype rapidly evolved resistance to insecticides in the United States (Horowitz et al. 2005; Khasdan et al. 2005).

The present study aimed to estimate the proportion of different biotypes in populations sampled from cotton fields not yet investigated, and also to evaluate their susceptibility to three neonicotinoids and one carbamate insecticide. Among the former, acetamiprid is the only insecticide usually sprayed on cotton fields in Burkina Faso.

2. Materials and methods

2.1. Whitefly populations

In 2007, at least 10,000 adults of *B. tabaci* were collected from cotton fields close to the villages of Sidéradougou, Houndé, Diébougou, Boromo, Solenzo, Datomo, Diabo, Diapaga, Pô and Bittou (Figure 1). The environmental factors and agricultural practices varied considerably in the different zones of the country (Table 1). In each area, after neonicotinoid treatments, *B. tabaci* adults were collected at random from 50 cotton plants using a mouth aspirator, then confined in a wooden rearing cage (50 cm by 35 cm by 35 cm) containing cotton seedlings, and returned to the laboratory within 2–5 h. Whiteflies of both sexes and different ages were tested the same day or the following day. At least, 30 individuals of *B. tabaci* were collected from fields and conserved in alcohol (80%) for molecular analysis in laboratory.

2.2. Insecticides

The following formulated insecticides were used for the bioassays: imidacloprid (Confidor 200 SL), provided by Bayer AG (Leverkusen, Germany), acetamiprid (Mospilan 200 SL) provided by Arysta Life Science (Noguères, France), thiamethoxam (Actara 240 SC) from Syngenta Crop Protection AG (Basel, Switzerland) and carbosulfan (Marshal 25 EC) provided by Syngenta Agro AG (Dielsdorf, Switzerland). Among the insecticides tested, only acetamiprid at 12 g active ingredient (a.i.) per hectare is used by farmers for cotton protection in Burkina Faso during the last treatments in combination with cypermethrin at 36 g a.i./ha (Gnankiné et al. 2007). The other insecticides may be used at any time and any dosages for protecting vegetables in growing areas.

2.3. Determination of Bemisia tabaci *biotypes or genetic groups*

Genomic DNA was extracted from each individual adult of *B. tabaci* in 26 μL of Nonidet P-40 extraction buffer (Delatte et al. 2005) and stored at −20°C. Biotypes were identified using a PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) based diagnostic assay. Briefly, in this method a fragment of the mitochondrial marker CO1 (cytochrome oxidase 1 gene sequences, *mtCOI*) gene is amplified by PCR (Frohlich et al. 1999) using universal COI primers C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and TL2-N-3014 (5′-TCCAATGCAC TAATCTGCCATATTA-3′) (Khasdan et al. 2005). The PCR assays were composed of 25 μL Platinum PCR SuperMix, 0.5 μL forward primer (10 pmol), 0.5 μL reverse primer (10 pmol) and 2 μL DNA template. The PCR products were then digested by the restriction endonucleases XapI (Fermentas) and/or BfmI (Fermentas), which generates clear polymorphism between biotypes B, MS, Q and Q1, Q2 or Q3 genetic groups. The PCR products were incubated with 10 U/μL XapI (Fermentas) at 37°C for 3 h before loading onto agarose gel (Henri et al. unpublished data).

2.4. Bioassay

A leaf dip bioassay method was performed based on previous studies (Rowland et al. 1991; Cahill et al. 1995).

Table 1. Frequencies of *Bemisia tabaci* biotypes related to environmental factors and agricultural practices in Burkina Faso.

Population	Geographic coordinates		n[1]	Climatic zones	Host plant	Vegetable cropping	Frequency of neonicotinoid used	Other Insecticides used[2]	Season	Biotype or genetic group (%)
Diapaga	1°49′11,28″W;	12°1,87’52,2"N	32	Sudanian	Cotton	absent	Low	OP, PYR	Rainy	Q1 (100)
Boromo	2°55’41,52"W;	11°47’52,8"N	24	Sudanian	Cotton	absent	Low	OP, PYR	Rainy	Q1 (55), ASL (45)
Houndé	3°31’12"W;	11°29’34,8"N	41	Sudanian	Cotton	absent	Low	OP, PYR	Rainy	Q1 (60), ASL (40)
Diébougou	3°15’0,0"W;	10°57’46,8"N	27	Sudanian	Cotton	Tomato, marrow	High	OP, PYR	Rainy and dry	Q1 (100)
Sidéradougou	4°15’12,6"W;	10°40’30"N	25	Guineo-sudanian	Cotton	Tomato, marrow, eggplant	High	OP, PYR	Rainy and dry	Q1 (100)
Pô	1°8’42"W;	11°9’54"N	33	Guineo-sudanian	Cotton	Tomato, marrow	High	OP, PYR	Rainy and dry	Q1 (70), ASL (30)
Bittou	0°18’12,84"W;	11°15’0,0"N	22	Sudanian	Cotton	Tomato, eggplant	Low	OP, PYR	Rainy	Q1 (100)
Diabo	0°18’2,52"W;	12°78’4,69"N	31	Sudanian	Cotton	Tomato	High	OP, PYR	Rainy and dry	Q1 (100)
Datomo	3°14’12,84"W;	11°54’54"N	28	Sudanian	Cotton	absent	Low	OP, PYR	Rainy	Q1 (100)
Solenzo	4°5’6"W;	12°10’37,2"N	32	Sudanian	Cotton	Tomato, eggplant	High	OP, PYR	Rainy and dry	Q1 (100)

[1]Number of individuals in the sample.
[2]This column indicates the use of organophosphate (OP) and pyrethroid (PYR).

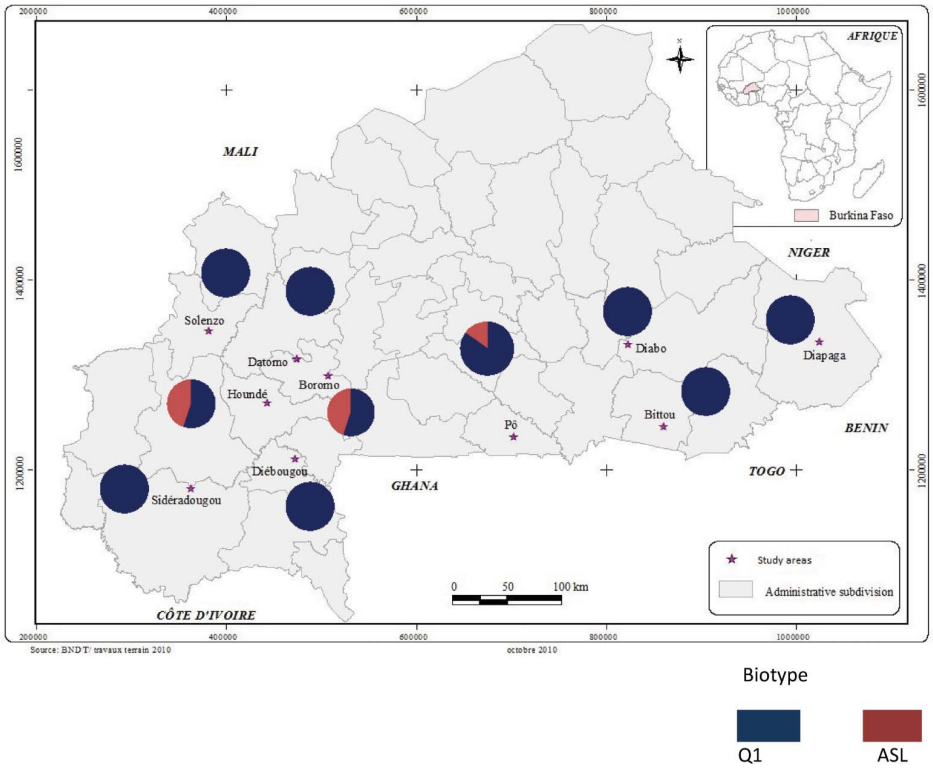

Figure 1. (Colour online) Map of study areas and prevalence of *Bemisia tabaci* biotypes in Burkina Faso.

For each insecticide, discs (35 mm in diameter) of cotton leaves were immersed for 10 s in seven aqueous solutions of various concentrations of insecticide, or in distilled water (controls). Leaf discs were air dried for 30 min. Discs were then positioned individually on an agar-coated (7 g L^{-1}) in a vial (45 mm diameter). Adults of *B. tabaci* (30 individuals of mixed sex) were removed from cotton leaves with a mouth aspirator, chilled and transferred into small plastic vials containing the treated leaf discs. Each vial was then sealed with a transparent ventilated lid. When adults recovered from chilling, vials were stored upside down and maintained at 25°C (± 2), 60% RH (± 5) and a 12 : 12 h light : dark photoperiod. An insect was considered alive if any sign of movement was observed. Mortality was recorded 24 h later. Three replicates were carried out for each concentration of insecticide and untreated controls. Mortality in the control was always < 10% and data from all bioassays were corrected for control mortality using Abbott's formula (Abbott 1925).

2.5. *Statistical analyses*

2.5.1. *Impact of geographical location on the distribution of biotypes*

The data collected were subjected to Fisher's exact test with simulated *P*-values based on 2000 replicates using R statistical software (http://www.R-project.org).

2.5.2. *Dose–response bioassays*

All bioassay replicates were combined for analysis. LC_{50} values were calculated with global optimization by simulated annealing (GOSA), available at http://bio-log.biz. This software tests the linearity of dose–mortality responses and provides the slope, lethal concentrations (LC_{50}'s) and 95% confidence limits. A population is considered to be significantly ($P < 0.05$) more (or less) resistant than another population when there is no overlap of the 95% confidence limits of the LC_{50}. The resistance ratios (RR) were calculated following the report of various LC_{50}'s on the LC_{50} of the most susceptible field population.

3. Results

3.1. *Biotype or genetic groups*

In our study, all *B. tabaci* from populations collected belonged to the Africa/Middle East/Asia Minor group (biotypes B, Q, ASL, Ms) (Figures 1 and 2). A significant relationship was found between the geographical range and the group distribution (Fisher's Exact Test, $P = 0.0005$). Q1 group was largely predominant according to various areas. It has been observed in Sidéradougou, Diébougou, Solenzo, Datomo, Diabo, Diapaga, and Bittou. The Q1 was found in sympatry with the ASL biotype in populations from Boromo, Houndé and Pô.

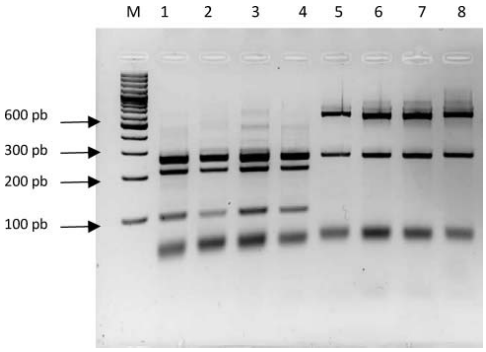

Figure 2. Representative gels showing ASL and Q1 biotypes collected in Burkina Faso. Lanes 1, 2, 3, 4: Q1 biotype; Lanes 5,6,7 8: ASL biotype, digested with *Xap*I. M: Hyper ladder IV.

3.2. *Resistance to insecticides*

Populations with only Q1 appeared to be more resistant to acetamiprid, thiamethoxam and carbosulfan (Table 2). Among the 10 *B. tabaci* populations tested, those living in sympatry (ASL and Q1) from Boromo, Houndé and Pô were in most cases more susceptible to the three neonicotinoids (Figure 1; Table 2). On the contrary, the highest neonicotinoid resistant populations were all from pure Q1, such as the populations from Diabo, Solenzo and Bittou which were highly resistant to acetamiprid, imidachloprid and thiamethoxam, respectively. In the absence of reference strain, the *B. tabaci* population from Houndé was used as reference to calculate the resistance ratio (RR) for neonicotinoids. Q1 populations from Diapaga, Boromo and Bittou exhibited some variation in their response towards acetamiprid compared to Houndé (RR_{50} 2 to 9), while highest resistance (RR_{50} 23 to 50) was observed in the populations from Sidéradougou (Q1), Po (Q1 and ASL), and Diabo (Q1). Q1 and ASL populations from Boromo and Po exhibited some variation in their response towards imidacloprid compared to Houndé (RR_{50} 1 to 2), while the highest resistance (RR_{50} 5 to 14) was observed in the Q1 populations from Datomo, Diebougou, and Solenzo. Q1 and ASL populations from Boromo and Po exhibited some variation in their response towards to thiamethoxam compared to Houndé (RR_{50} 2 to 4), while the highest resistance (RR_{50} 13 to 33) was observed in the Q1 populations from Diabo, Solenzo and Bittou.

As for neonicotinoids, the three *B. tabaci* populations from Boromo, Houndé and Pô, where ASL and Q1 lived in sympatry, were among the most susceptible to carbosulfan (Figure 1; Table 2). In the absence of reference strain, the *B. tabaci* population from Boromo was used as a reference to calculate the resistance ratio (RR) for carbosulfan. The highest carbosulfan resistant

Table 2. Toxicity of different insecticides against field populations of *Bemisia tabaci* collected from cotton fields in Burkina Faso (West Africa) using leaf dip bioassays.

Insecticide	Population	Collection date	N^a	Slope ± SE	LC_{50} (mg L^{-1})	Confidence limits (95%)	RR_{50}[b]
Acetamiprid	Diapaga	11/14/2007	1085	1.2 ± 0.3	4.4 c	2.11–7.17	8.8
	Boromo	11/28/2007	1440	1.3 ± 0.4	1.4 d	0.03–2.50	2.8
	Houndé	11/30/2007	840	1.8 ± 0.7	0.5 d	0.19–1.4	–
	Diébougou	11/30/2007	960	1.28 ± 0.1	7.8 c	4.20–10.50	15.6
	Sidéradougou	12/12/2007	1240	2 ± 0.8	11.9 b	10.12–16.00	23.8
	Pô	09/11/2007	720	1.8 ± 0.6	12.14 b	9.00–15.00	24.28
	Bittou	11/11/2007	728	1.35 ± 0.2	3.5 c	2.10–5.10	7
	Diabo	11/13/2007	1096	1.61 ± 0.3	25 a	20.50–32.1	50
	Datomo	12/06/2007	965	1.9 ± 0.6	5.6 c	3.68–8.50	11.2
	Solenzo	9/15/2007	968	1.27 ± 0.1	7.4 c	4.1–11.10	14.8
Imidacloprid	Diapaga	11/14/2007	965	0.37 ± 0.1	22.5 b	20.00–25.00	3.9
	Boromo	11/28/2007	1200	0.43 ± 0.2	6.3 c	4.24–8.80	1.09
	Houndé	11/30/2007	967	0.41 ± 0.3	5.8 c	3.21 – 9.00	–
	Diébougou	11/30/2007	960	1.31 ± 0.5	34 b	28.70–40.90	5.86
	Sidéradougou	12/12/2007	1240	0.35 ± 0.1	12 bc	9.5–14.60	2.1
	Pô	09/11/2007	1220	0.38 ± 0.1	6.3 c	4.2–8.1	1.08
	Bittou	11/11/2007	828	0.36 ± 0.2	11 bc	8.50–14.00	1.9
	Diabo	11/13/2007	1096	0.29 ± 0.1	17 bc	14.00–20.50	2.9
	Datomo	12/06/2007	1225	1.33 ± 0.7	31.3 b	21.00–40.00	5.4
	Solenzo	9/15/2007	1350	1.8 ± 0.8	80 a	70.7–90.00	13.8
Thiamethoxam	Diapaga	11/14/2007	1085	1.36 ± 0.05	4 c	2.1–5.50	2.7
	Boromo	11/28/2007	1440	1.32 ± 0.02	3.4 c	1.00–5.70	2.3
	Houndé	11/30/2007	840	1.29 ± 0.1	1.5 c	0.1–3.51	–
	Diébougou	11/30/2007	960	1.90 ± 0.1	23 b	18.00–28.00	15.3
	Sidéradougou	12/12/2007	1.24	1.34 ± 0.5	37 a	32.50–42.00	24.7
	Pô	09/11/2007	720	1.35 ± 0.2	6.4 c	3.18–9.40	4.3
	Bittou	11/11/2007	728	2.71 ± 0.3	49.5 a	42.00–58.00	33
	Diabo	11/13/2007	1096	1.49 ± 0.1	18.6 b	12.15–23.6	12.4
	Datomo	12/06/2007	965	1.44 ± 0.1	21 b	18.4–24.00	14
	Solenzo	9/15/2007	968	2.60 ± 0.4	45 a	38.12–52.00	30
Carbosulfan	Diapaga	11/14/2007	1065	1.8 ± 0.3	7.80 d	5.05–9.80	1.1
	Boromo	11/28/2007	1095	1.1 ± 0.4	7.4 d	4.84–10.60	–
	Houndé	11/30/2007	740	1.4 ± 0.6	18.3 c	12.90–24.5	2.5
	Diébougou	11/30/2007	560	1.5 ± 0.3	28 c	25.30–32.10	3.8
	Sidéradougou	12/12/2007	569	1.34 ± 0.9	101 a	95.40 – 109.0	13.6
	Pô	09/11/2007	880	1.43 ± 0.2	34 c	27.90–42.80	4.6
	Bittou	11/11/2007	960	1.1 ± 0.3	10 d	6.05–14.00	1.4
	Diabo	11/13/2007	589	2.46 ± 0.7	65.7 b	59.80–70.20	8.9
	Datomo	12/06/2007	965	2.5 ± 1.0	76.6 b	69.80–82.00	10.4
	Solenzo	9/15/2007	968	2.32 ± 0.8	50 b	46.5–55.00	6.8

[a] N = number of whiteflies tested; [b] RR: Resistance Ratio = LC_{50} field population / LC_{50} Houndé population or Boromo population. For each insecticide, LC_{50}s followed by the the same letter are not significantly different.

populations were observed in pure Q1 populations such as Datomo and Sideradougou (RR_{50} 7 to 14). Q1 populations from Houndé, Diebougou and Po exhibited some variation in their response towards carbosulfan compared to Boromo (RR_{50} 2 to 5).

4. Discussion

According to the determination of biotypes, the populations of *B. tabaci* tested belonged to the Africa/Middle East/Asia Minor group (biotypes B, Q, ASL, Ms). Biotypes Q and Africa-SL were identified in MED species (Boykin et al. 2007; De Barro et al. 2011). In the Q biotype, three groups were recently designated as Q1, Q2 (Chu et al. 2008) and Q3 (Gueguen et al. 2010). Gnankiné et al. (2012) showed that the Q1 was predominant in cotton fields and is probably progressively displacing the African ASL biotype. Q1 originating from the Mediterranean region is generally considered to be invasive, like the B biotype. It is the real *B. tabaci* (Tay et al. 2012), and has now begun its own global invasion spreading from its Mediterranean home range to at least 10 different countries (Dalton 2006; De Barro et al. 2011).

Our results confirmed the resistance of *B. tabaci* populations from Burkina Faso to neonicotinoids (Houndété et al. 2010a) and showed resistance to one carbamate. The resistance ratios (RRs) showed a variation between the populations from different localities and genetic groups. It

varied from 4 to 50 for the neonicotinoids and from 2 to 14 for the carbamate. The resistance ratios for the neonicotinoids were much higher than those obtained three years ago by Houndété et al (2010a) in populations collected in Soumousso and Tiara, Burkina Faso (RR = 3 to 8). The increase of the neonicotinoid resistance ratio might be explained by the use of acetamiprid in the two last insecticide sprays on cotton recommended by the ginning companies who provide insecticides, as credit, to cotton farmers. However, a part of these insecticides is deflected to the black economy. That is the reason why most of insecticides used for protecting vegetables come from cotton (Ahouangninou et al. 2012). On the contrary, the low resistance level of *B. tabaci* to carbosulfan may be explained by the non-use of this insecticide for cotton protection. Up to now, to control whiteflies, farmers use acetamiprid that was progressively introduced in 2000 as a substitute for organophosphates, which become inefficient due to acetylcholinesterase resistance (Houndété et al. 2010b).

Our results suggest that the neonicotinoid resistance status of *B. tabaci* is linked to the biotype, especially to Q1. Indeed, the highest resistance factors to acetamiprid and thiamethoxam were recorded in the Q1 populations. One noteworthy finding is that the correlation between the neonicotinoid resistance and the biotype was not clearly demonstrated. However, the most susceptible *B. tabaci* populations were collected in Boromo and Houndé. These populations were composed of Q1 and ASL individuals with quite the same prevalence. In Benin, previous studies have already identified neonicotinoid-susceptible populations as belonging to the ASL biotype compared with heterogeneous populations from Burkina Faso that were resistant (Gueguen et al. 2010; Houndété et al. 2010b; Gnankiné et al. 2012). The susceptibility of *B. tabaci* population from Diapaga compared to other areas with pure Q1 biotype could be due to the low insecticide pressure coupled with the absence of vegetable crops around. This result suggests that individuals from Q1 biotype should be always susceptible to neonicotinoid, which does not exclude resistance to other insecticides. Recently, L. Mouton et al. (unpublished data) have demonstrated the presence of *kdr* and *Ace1-R* genes in this population. In Israel, Horowitz et al. (2003) showed that the Q biotype was predominant in areas where resistance to pyriproxyfen evolved rapidly. Cases of resistance of *B. tabaci* Q populations to pyrethroids were demonstrated in Greece by Roditakis et al. (2009). Moreover, in southern Spain, the Q biotype was linked with high and cross-resistance to neonicotinoids (Guirao et al. 1997; Nauen et al. 2002). After a few years, the Q had almost displaced the B, especially in southern Spain (Simón et al. 1999) probably because of the increased use of neonicotinoids against whiteflies. Our results confirmed also the cross-resistance between neonicotinoids, as the Sideradougou population, for example, was highly resistant to both acetamiprid (23.8-fold) and thiametoxam (24.7-fold). The extreme resistance to acetamiprid in Diabo Q1 populations (50-fold) is probably due to the extensive use of this insecticide. As far as imidachloprid is concerned, the Solenzo population appears to be the only one displaying highest resistance (13.8-fold). Karunker et al. (2008) showed that cytochrome P450 CYP6CM1 was implied in the metabolic resistance of neonicotinoids and appears to be specific for imidachloprid (Roditakis et al. 2011). It has been demonstrated that overexpression of CYP6CM1 is associated with high levels of imidacloprid resistance in *B. tabaci* (Karunker et al. 2008).

The current status of *B. tabaci* resistance to neonicotinoid insecticides from West Africa requires urgent attention if neonicotinoids really did select the invasive Q biotype. These results show again the negative effect of using continuously the same molecule, or the same family of insecticides, year after year until inefficiency. Moreover, our results suggested the selection of a new multi-resistant phenotype may threaten the production of cotton or vegetables in the future. Indeed, the spread of a potentially more prolific vector for begomovirus virus will surely lead to a crisis in the production of Solanaceae and especially tomatoes by small-scale farmers in West and Central Africa (Hanafi 2000).

The use of genetically modified *Bt* cotton in Burkina Faso since 2008 could be one way to avoid the selection of *B. tabaci*-resistant populations by reducing insecticide use, which naturally enhances the impact of natural enemies. The suppression of insecticide treatments against whiteflies that are systematically applied before harvest could have a significant impact on sticky cotton. But this problem can easily be solved by harvesting cotton not too late after boll opening or by controlling the degree of stickiness before the ginning process.

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Research Reports

Microclimate Modification Using Eco-friendly Nets for High-quality Tomato Transplant Production by Small-scale Farmers in East Africa

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Summary. **Unfavorable environmental conditions, pests, and viral diseases are among the major factors that contribute to poor growth and quality of tomato (*Solanum lycopersicum*) seedlings in tropical areas. Improving crop microclimate and excluding insects that transmit viruses may improve transplant quality and yield in production fields. This study was carried out in two seasons at the Horticulture Research and Teaching Field of Egerton University in Njoro, Kenya, to investigate the effects of agricultural nets herein called eco-friendly nets (EFNs) on germination and performance of tomato seedlings. Tomato seeds were either raised in the open or under a permanent fine mesh net (0.4-mm pore diameter). Eco-friendly net covers modified the microclimate resulting in significantly higher day temperatures and relative humidity, compared with the open treatment. Nets increased temperature and relative humidity by 14.8% and 10.4%, respectively. Starting seeds under a net advanced seedling emergence by 2 days and resulted in higher emergence percentage, thicker stem diameter, more leaves, and faster growth leading to early maturity of seedlings and readiness for transplanting. Netting improved root development by increasing root quantity and length. Stomatal conductance (g_S) and estimates of chlorophyll content were higher in seedlings under net covering compared with those in the noncovered control treatment. Insect pests and diseases were also reduced under net covering. The use of the net in the production of tomato transplants presented a 36.5% reduction in the cost of seeds, through improved emergence and reduced pest damage. All other factors held constant, healthy and quality transplants obtained under a net covering also translate into better field performance; hence, increasing economic returns for commercial transplants growers, as well as for tomato farmers. Results of this study suggest that EFNs can be customized not only for their effective improvement on growth and quality of tomato transplants but also for their pest and disease management in the nursery alone or as a component of integrated pest and disease management.**

Tomato is a popular and versatile food crop grown and consumed all over the world and is the second most important commercial vegetable crop to potato (*Solanum tuberosum*) (Naika et al., 2005). It is one of the most widely cultivated vegetable crops in Africa, including Kenya. It is grown for home consumption in almost every homestead across the nation serving as an important source of vitamins and a cash crop for both small- and medium-scale commercial farmers. Apart from vitamins, tomato is also rich in lycopene, an antioxidant, which purportedly fights free radicals that interferes with normal cell growth and activity and thus reducing cancer, heart diseases, and premature aging (Nkondjock et al., 2005). The fruit also contain significant amounts of minerals and fiber. In Kenya, tomatoes always are in high demand both for fresh consumption and processing (Mungai et al., 2000).

Tomato are established by direct seeding or from transplants (Long and Cantliffe, 1975). However, reports have indicated that use of transplants is preferred because of higher plant survival, faster establishment, improved plant uniformity, earlier maturity, and reduced cost of production than direct-seeded plants (Leskovar and Cantliffe, 1994). Whether started directly or by transplants, optimum growth and quality of tomato is dependent on the environment. As a result, researchers have modified the environment to favor the growth of the plant (Caliman et al., 2010; Weerakkody, 1998). Tomato seeds are expensive and farmers in developing countries cannot afford poor germination as a result of unfavorable growing conditions [Horticultural Crop Development Authority (HCDA), 2006]. In addition, tomato seedling production in many parts of sub-Saharan Africa is faced with major challenges like unfavorable weather marked by limited or unreliable rainfall, high or low temperatures depending on the season, as well as severe pest infestation. These challenges are exacerbated by the fact that most tomato growing is by small-scale, resource-poor farmers who cannot afford expensive protected greenhouse crop production technologies. They must therefore produce their seedlings and crop in open fields subject to unfavorable growing conditions. The result of this scenario has been an increased cost of seed per unit area due to poor germination and pest damage. Insect pests like silverleaf whitefly (*Bemisia tabaci*), potato aphid (*Macrosiphum euphorbiae*), and western flower thrips (*Frankliniella occidentalis*) that transmit viral diseases are also experienced under such conditions. In a study conducted in Israel, Berlinger et al. (2002) reported effective management of tomato yellow leaf curl virus through physical exclusion of

silverleaf whiteflies using the nets. On the other hand, the use of protected seedling culture has been shown to improve crop yields. In tomato, higher yields may be achieved with protected than nonprotected seedlings (El-Aidy and Sidaros, 1996).

In an effort to enhance emergence and growth of seedlings, growers normally use mulching as a cultural practice to raise seedlings and a microclimate modification strategy. However, some mulch material, especially organic mulch which is commonly used by small-scale resource-poor farmers in sub-Saharan Africa, harbors various seedling pests and diseases, resulting in additional pesticide and fungicide costs. Many concerns have been raised on the safety of these chemicals to humans and to the environment, and on their cost (HCDA, 2006; Martin et al., 2006; Ministry of Agriculture and Rural Development, 2003). Excessive use of these chemicals also results in the development of resistance in plant pests and diseases. Thus, the need to look into effective and safer eco-friendly methods of control is increasing.

Protected culture is predominantly used in temperate regions where seasonal agro-climatic changes restrict year-round cultivation of crops under open-field conditions. Due to this advantage, Iqbal (1987) recommended its applicability in tropical environments especially for cultivating high value crops such as tomato during the rainy season. One possibility of protected culture could be the use of EFNs. Nets have been used to protect crops from excessive solar radiation or other environmental hazards in developed countries (Majumdar, 2010). Use of EFN in protected cultivation has been tested and proven to be effective against certain pests of cabbage [*Brassica oleracea* var. *capitata* (Licciardi et al., 2007; Martin et al., 2006)], on alternaria blight (*Alternaria brassicae*), and on black rot (*Xanthomonas campestris* pv. *campestris*) of cauliflower (*B. oleracea* var. *botrytis*) seedlings (Kashyap and Dhiman, 2010). Also noted was reduction in pesticide use as a result of covering the crop with nets. In line with these observations, the current study aimed at investigating the effects of EFN on germination and performance of tomato seedlings in open fields.

Materials and methods

Experimental site

The study was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro, Kenya, in two seasons (Mar. to Apr. 2011 and Sept. to Oct. 2011). The field lies at latitude 0°23′S and longitude 35°35′E in the Lower Highland III (LH3) Agro-Ecological Zone at an altitude of ≈2238 m above sea level. Average maximum and minimum temperatures range from 19 to 22 °C and from 5 to 8 °C, respectively, with a total annual rainfall of 1200 to 1400 mm and predominantly andosols soils with a pH of 6.0 to 6.5 (Kassilly, 2002).

Planting material, experimental design, and treatments

'Rio Grande' tomato seeds (Kenya Seed Co., Kitale, Kenya) were used in the experiment. 'Rio Grande' is a cultivar with a determinate growth habit and was chosen based on the recommendation of the Kenya Agricultural Research Institute.

The experimental design was a randomized complete block with two treatments and five replications. Treatments consisted of tomato seedlings produced either under a fine mesh net (0.4-mm pore diameter) (AgroNet; A to Z Textile Mills, Arusha, Tanzania) or in the open (control). Immediately after sowing, metal arches (U-shape) of 0.5-m height and 1-m width were placed over the plots and secured in the soil. These arches were then covered with netting, which was pegged at each corner to minimize wind interference.

Land preparation and maintenance practices

Experimental plots were manually prepared (common practice used by growers in the region) to ≈30-cm depth with final preparation using a fine tooth rake. Beds of 1 × 2 m were prepared and raised to 15-cm height. Thirteen rows, 1-m long and 15-cm apart, were prepared per bed. Tomato seeds were sown at a spacing of 1 cm within the drills. For the control treatments, dry grass was used for mulching and removed after first seedling emergence, as a standard practice used by local farmers. Thereafter, watering was done manually to field capacity whenever necessary ensuring that an equal amount was applied to each plot. Weeding and all other management practices were applied uniformly in all plots.

Data collection

Microclimate. A thermo-hygrometer (HM9; Shangai Precision and Scientific Instrument Co., Shangai, China) was mounted at the center of each bed to monitor relative humidity and air temperature. Data were collected and recorded on a daily basis in the morning (8:00 AM), at noon, and in the evening (4:00 PM) and averaged to obtain the daily average relative humidity and temperature.

Leaf g_S and chlorophyll content. Leaf g_S ($mmol \cdot m^{-2} \cdot s^{-1}$) and estimates of chlorophyll content in chlorophyll concentration index units (CCIs) were determined on the

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

two lower true leaves of 20 randomly selected tomato seedlings from the central rows of each plot starting 21 d after planting (DAP) through the fifth week on a weekly basis. A steady state leaf porometer (SC-1; Decagon Devices, Pullman, WA) and chlorophyll content meter (CCM-200 plus; Opti-Sciences, Tyngsboro, MA) were used, respectively.

Seedling emergence. The number of days from planting to first seedling emergence was recorded for every treatment. Thereafter, seedling numbers were counted at a 2-d interval for 1 week following first emergence, and the progressive emergence percentages were computed for each treatment.

Seedling growth. Twenty central tomato seedlings were randomly selected and tagged for data collection on the first day of seedling growth data collection. From these plants, seedling height, stem diameter, and number of leaves were determined on a weekly interval. During seedling harvesting, 10 seedlings were randomly selected, carefully uprooted, the roots were washed with clean water, the length of the main root was measured using a ruler, and the number of lateral roots were counted.

Insect pest population and disease incidences. The species and number of insect pests were counted on a weekly basis from two leaves of 20 central tomato seedlings selected at random in each plot at each data collection date beginning 1 week after emergence. From the same seedlings, those that showed disease symptoms were also counted and recorded to determine percent disease incidence.

Seed requirement and cost per unit area. From the percentage emergence, seed requirement and cost of seed per hectare were estimated for the different treatments.

Data analysis

The Proc univariate procedure of SAS (version 9.1; SAS Institute, Cary, NC) was used to control for normality of the data before analysis. Data were then subjected to analysis of variance (ANOVA) at $P \leq 0.05$ using GLM. An initial analysis using season as a factor in the model showed no significant difference in season and season × treatment interaction for most parameters. Based on this, data for the two seasons were pooled together and analyzed using the statistical model: $\Upsilon_{ij} = \mu + \beta_i + \alpha_j + \varepsilon_{ij}$, where Υ_{ij} is the tomato seedling response, μ is the overall mean, β_i is the ith blocking effect, and α_j is the effect due to the jth net covering. Significantly different means were separated using least significant difference (LSD) at $P \leq 0.05$.

Results

Microclimate modification. Mean daily temperature and relative humidity were significantly higher under the netting treatment compared with the control throughout the study (Fig. 1). The average daily temperature was 26.8 °C under the net and 23.3 °C in the control treatments reflecting a 15% increase under the nets. Netting also resulted in a higher relative humidity averaging 58.2% compared with 52.7% for the control. Light was not measured in the nursery but in other experiments the nets reduced light quantity (37.3 mol·d^{-1})

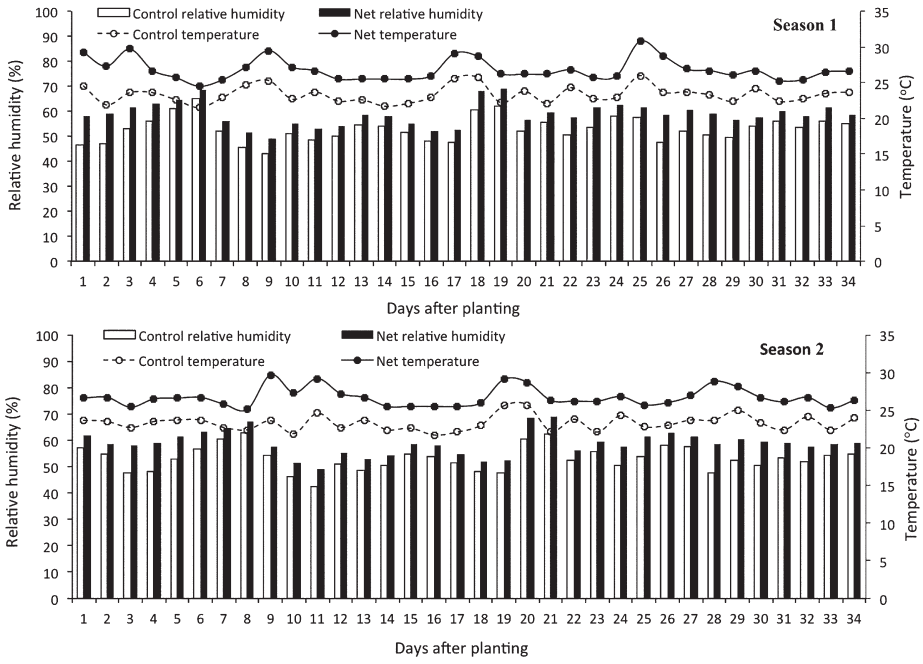

Fig. 1. Effect of eco-friendly net on daily air temperature and relative humidity during tomato transplant production in season 1 (Mar. to Apr. 2011) and season 2 (Sept. to Oct. 2011) at Egerton University, Njoro, Kenya; (1.8 × °C) + 32 = °F.

compared with the open treatment (40.1 mol·d^{-1}).

Seedling physiology. Use of EFN significantly enhanced leaf g_S and chlorophyll content (Table 1). Leaf g_S increased significantly as seedling age increased both under the control and nets, with netting recording the highest g_S at 35 DAP (352.2 mmol·m^{-2}·s^{-1}), compared with 267.9 mmol·m^{-2}·s^{-1} in the control treatment.

Leaf chlorophyll content also increased significantly as seedlings aged both under control and nets. During the final day (35 DAP), the net recorded 34.0 CCI while the control had 23.3 CCI. The CCI values are estimates of leaf chlorophyll concentration with higher values indicating higher chlorophyll content.

Seedling emergence and Growth. Seedling emergence was enhanced by net covering (Table 2). On average, seeds sown under netting took 6 d from planting to first emergence compared with 8 d under control depicting a 25% reduction in time for seedling emergence.

Seeds sown under netting had a higher emergence percentage compared with the control throughout the evaluations. By 7 DAP, 61.9% of sown seeds had emerged in the nets compared with only 7.2% for the control. By 11 DAP, the nets had 96.2% of sown seeds emerged compared with 57.1% in the control treatment.

Seedling growth was enhanced under netting (Fig. 2). Seedling height showed a steady increase during all evaluation dates for both treatments. Seedlings under the net were significantly taller compared with the control seedlings with means of 12.0 and 4.7 cm, respectively, during the last data collection day (31 DAP).

The number of leaves differed significantly between treatments. Seedlings under netting had more leaves compared with those in the control at all dates. After 10 DAP, the number of leaves in the control treatment remained relatively constant with 2.4 leaves per seedling. However, seedlings under the nets produced more leaves at 10 and 17 DAP with 2.5 and 3.6 leaves per seedling, respectively.

Control seedlings were thinner as depicted by the smaller stem diameters compared with those raised under netting. There was a progressive increase in stem thickness from 10 DAP to 31 DAP under both netting and the control. At 31 DAP, stems of netted seedlings were 4.8-mm thick, whereas control seedlings were 3.4-mm thick.

Netting improved seedling root development. Longer roots (8.8 cm) were obtained under the net compared with 8.0-cm mean root length for the control (Table 3). More lateral roots were also observed under the net compared with the control. Mean lateral root number per seedling were 26.1 cm and 17.0 cm for the seedling under the nets and the control, respectively.

Insect pest population and disease incidences. Use of EFN helped manage various pest and diseases (Table 4). The number of leafminers (*Lyriomyza* sp.), cotton bollworms (*Helicoverpa armigera*), onion thrips (*Thrips tabaci*), mites (*Tetranychus* sp.), silverleaf whiteflies, and aphids (*Aphis* sp.) were significantly lower under the net covering with a mean of 0.1, 0.1, 0.2, 0.1, 0.1, and 0.2 pests per plant, respectively, over the study period compared with 0.4, 0.4, 2.9, 2.3, 1.8, and 3.6 pests per plant, respectively, under the control.

The major disease observed in the experiment was late blight (*Phytophthora infestans*). The number of seedlings with disease symptoms was significantly lower under the netting treatments with a mean of 4.3% compared with 44.5% in the control.

Seedling requirement and cost per unit area. Based on seedling emergence percentage, there was a potential of reducing seed requirement per unit area when a net covering was used in nursery seedling production compared with when seedlings were produced in the open (Table 5). Nets had a final emergence of 96.2% while the control had only 59.6% emergence. Using the recommended spacing for 'Rio Grande' of 1 × 0.5 m, this would imply that a grower planting 1 ha of tomato would have to buy 770 more seeds (3.9%) when using the nets to raise the seedlings before transplanting while one starting the seedlings in an open nursery would have to pay for 8080 more seeds (40.4%) to

Table 1. Change in physiological characteristics of tomato seedlings as influenced by eco-friendly net covering at Egerton University, Njoro, Kenya. The values shown are averaged across two seasons, Mar. to Apr. 2011 and Sept. to Oct. 2011.

	g_S (mmol·m^{-2}·s^{-1})			Chlorophyll content (CCI)[y]		
	DAP (d)[x]					
Treatment[z]	21	28	35	21	28	35
Control	178.3 b[w]	222.5 b	267.9 b	11.0 b	17.0 b	23.3 b
Net	275.2 a	322.2 a	352.2 a	18.4 a	27.0 a	34.0 a
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD[w]	5.5	1.1	0.8	0.4	0.7	0.3

[z]Control treatment was mulched with dry grass as a standard practice used by farmers in the region. Net treatment was covered with a fine mesh net of 0.4-mm (0.016-inch) pore diameter.
[y]Chlorophyl content index is an estimate of chlorophyll content.
[x]Days after planting.
[w]Means followed by the same letter within a parameter and column are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Table 2. Changes in emergence of tomato seedlings as influenced by eco-friendly net covering at Egerton University, Njoro, Kenya. The values shown are averaged across two seasons, Mar. to Apr. 2011 and Sept. to Oct. 2011.

		Emergence (%)		
		DAP (d)[y]		
Treatment[z]	Time to first emergence (d)	7	9	11
Control	8 a[x]	7.2 b	55.5 b	57.1 b
Net	6 b	61.9 a	88.2 a	96.2 a
P value	<0.0001	<0.0002	0.0009	<0.0001
LSD[x]	0.9	11.2	10.3	3.8

[z]Control treatment was mulched with dry grass as a standard practice used by farmers in the region. Net treatment was covered with a fine mesh net of 0.4-mm (0.016-inch) pore diameter.
[y]Days after planting.
[x]Means followed by the same letter within a parameter and column are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Table 4. Effect of eco-friendly net on pest population and disease attack on tomato seedling in at Egerton University, Njoro, Kenya. The values shown are averaged across two seasons, Mar. to Apr. 2011 and Sept. to Oct. 2011.

Treatment[z]	Leafminers (no./plant)	Cotton bollworms (no./plant)	Onion thrips (no./plant)	Mites (no./plant)	Silverleaf whiteflies (no./plant)	Aphids (no./plant)	Disease (%)
Control	0.4 a[y]	0.4 a	2.9 a	2.3 a	1.8 a	3.6 a	44.5 a
Net	0.1 b	0.1 b	0.2 b	0.1 b	0.1 b	0.2 a	4.3 b
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD[y]	0.1	0.1	0.3	0.2	0.1	0.1963	1.3553

[z]Control treatment was mulched with dry grass as a standard practice used by farmers in the region. Net treatment was covered with a fine mesh net of 0.4-mm (0.016-inch) pore diameter.
[y]Means followed by the same letter within a parameter and column are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Table 5. Effects of eco-friendly net covering on tomato seed emergence, seed requirement, and percentage increase in seed cost at Egerton University, Njoro, Kenya. The values shown are averaged across two seasons, Mar. to Apr. 2011 and Sept. to Oct. 2011.

Treatment[z]	Emergence (%)	Seeds (no./ha)[y]	Cost increase (%)[x]
Control	59.6	28,080	40.4
Net	96.2	20,770	3.9

[z]Control treatment was mulched with dry grass as a standard practice used by farmers in the region. Net treatment was covered with a fine mesh net of 0.4-mm (0.016-inch) pore diameter.
[y]1 seed/ha = 0.4047 seed/acre.
[x]Based on 100% emergence of tomato seedlings, which required 20,000 seeds/ha according to the spacing of 'Rio Grande' [1 × 0.5 m (3.28 × 1.64 ft)].

could have enhanced uptake of nutrients such as potassium and nitrogen, thereby favoring leaf g_S and chlorophyll content.

Higher transplant success is ideal for any grower. Tomatoes grown inside the nets emerged earlier than those under the control by 2 d. In addition, the final emergence percentage was higher under the net compared with the control. Temperature and soil moisture are among essential factors for successful emergence. The modified internal temperatures and higher soil moisture conditions observed under the nets in the current study could have favored biochemical and physiological processes necessary for germination within the seeds resulting early and higher emergence of seedlings. These findings are in line with results of a previous study on advantages of growing tomatoes under protected culture (Weerakkody and Peiris, 1998). Upon emergence, seedlings under netting showed a relatively better growth than the control seedlings. In general, seedlings in the open (control) required 7 d or more to achieve a similar height to those under the nets. Similarly, seedlings under netting obtained a given leaf number 7 d earlier than the control seedlings. Seedlings under the nets had a thicker stem diameter compared with the control by achieving a given diameter 14 d earlier. These observations agree with the findings of Weerakkody et al. (1999), who reported enhanced growth of protected tomatoes compared with a control. Better root development (length and number of roots) was also observed under netting treatments compared with the control. These results can be attributed to the microclimate created under the net covering that could have favored better physiological development of seedlings, which is consistent with observations of Adams et al. (2001).

Use of net covering in seedling production considerably reduced populations of leafminers, cotton bollworms, onion thrips, mites, silverleaf whiteflies, and aphids. The net covering used offered a physical barrier excluding many pests, hence lowering the population on the seedlings. In addition, the white nets used could be also a visual barrier for flying pests such as leafminers, onion thrips, moths (Lepidoptera), and silverleaf whiteflies distracting their feeding and mating, hence lowering their population under the nets. In their field tests, Licciardi et al. (2007) and Martin et al. (2006) observed a delay in the infestation of cabbage by aphids under netting. The reduction of the silverleaf whitefly population in seedlings could reduce and delay the risk of begomovirus transmission such as tomato yellow leaf curl virus to the plant (Berlinger et al., 2002). Higher relative humidity recorded under the netting could also affect the feeding habit of sucking pests, such as silverleaf whitefly and aphids, and consequently lowering their population under netting. Similar results have also been reported in previous studies (Berlinger et al., 2002). In addition, the nets effectively managed late blight incidence compared with the control. Temperature and relative humidity are necessary for the development of the crop and disease pathogens. Most fungal diseases are effective at a temperature range of 25 to 28 °C and relative humidity above 80%. Even though this temperature range was achieved under the nets, relative humidity was not high enough to favor spore germination that probably reduced disease development under the nets. These results confirm those of Kashyap and Dhiman (2010) while working with nylon nets in the management of alternaria blight and black rot in cauliflower seedlings. Although the incidence of viral diseases was not evaluated in the nursery and subsequent field evaluation, it is likely that the use of netting could provide significant benefits in management of those diseases.

Every farmer would prefer to reduce production costs while maximizing profits; the use of netting in seedling production showed a greater reduction in cost than the control. A farmer using nets for protection may save on the cost of buying seed by ≈36% on average. This is supported by higher transplant success under the nets compared with the control requiring less seed and potentially increasing economic returns. However, a full economic analysis of the technology including net cost, net management cost, pesticides, and application costs would need to be conducted. Findings

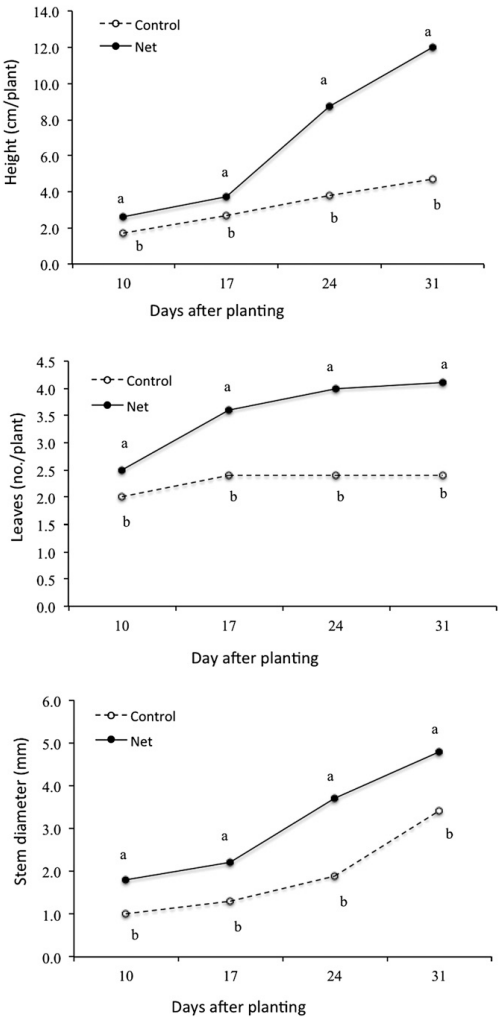

Fig. 2. Effect of eco-friendly net on seedling height, number of leaves, and stem diameter evolution during tomato transplant production in season 1 (Mar. to Apr. 2011) and season 2 (Sept. to Oct. 2011) at Egerton University, Njoro, Kenya. The values shown are averaged across the two seasons. For a specific parameter, data points within the same date with the same letter are not significantly different at $P \leq 0.05$; 1 cm = 0.3937 inch, 1 mm = 0.0394 inch.

compensate the reduction in stand establishment. This represents a 36.5% reduction in seed cost under the nets compared with the control.

Discussion

Netting effectively modified the microclimate around the growing seedlings. There was an average increase in daily temperature by ≈3.5 °C as a result of covering the seedlings with netting. The use of netting and other types of covering has been shown to restrict air movement around the growing seedlings resulting in higher temperatures (Majumdar, 2010; Nair and Ngouajio, 2010). Similar results have been reported by Shahak et al. (2004), using nets of different colors, and Weerakkody and Peiris (1998), on polyethylene cover. Relative humidity also was significantly higher inside the nets compared with the control registering ≈10% mean increase. Reduction in air movement, evapotranspiration, high temperature, and shading under netting probably caused the increase in relative humidity. These findings are in contrast with those of Parvej et al. (2010), who reported lower relative humidity under polyethylene-covered tomatoes. This is an indication that the impact of nets on microclimate may vary depending on local environmental conditions. Therefore, site-specific studies should be conducted to support recommendations on using the nets in major agro-ecological regions.

Seedlings under the nets had better physiological performance compared with those grown outside. Higher leaf g_S was recorded on seedlings inside the nets with an average increase of 42% compared with g_S of the control seedlings. Seedlings inside the nets showed higher chlorophyll content compared with the control seedlings registering a 55% increase in CCI. Tomato is a warm-season crop, thus temperature increases would favor several physiological and biochemical processes like photosynthetic enzyme activity, g_S, carbon dioxide diffusion, and photoassimilate translocation that would in turn lead to improved seedling growth. Similar observations have also been reported by Adams et al. (2001). Elevated air temperatures inside the net treatments together with improved moisture status and root development

Table 3. Effects of eco-friendly net covering on root development of tomato seedlings at Egerton University, Njoro, Kenya. The values shown are averaged across two seasons, Mar. to Apr. 2011 and Sept. to Oct. 2011.

Treatment[z]	Root length (cm/plant)[y]	Roots (no./plant)
Control	8.0 b[x]	17.0 b
Net	8.8 a	26.1 a
P value	<0.0001	<0.0001
LSD[x]	0.1	1.5

[z]Control treatment was mulched with dry grass as a standard practice used by farmers in the region. Net treatment was covered with a fine mesh net of 0.4-mm (0.016-inch) pore diameter.
[y]1 cm = 0.3937 inch.
[x]Means followed by the same letter within a parameter and column are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

of this study show the immense potential of using nets as an eco-friendly strategy in protecting seedlings from pests and diseases while enhancing their growth and quality as a result of the modified microclimate. In light of these findings, we recommend that the technology be tested on other crops and also with different mesh sizes and colors. It would also be extremely important to test this technology in each agro-ecological region before recommendations to growers since ambient conditions affect the changes in the microclimate that results from net use.

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